

THE BOTANICAL REVIEW

VOL. II

MAY, 1936

No. 5

STATISTICAL ECOLOGY

ERIC ASHBY

The University, Bristol, England

INTRODUCTION

It is almost inappropriate to publish an article on statistical ecology in this journal which records botanical progress, for many applications of statistics to the analysis of vegetation must be regarded as signs of decadence rather than of progress. Most biologists are not mathematically minded and the introduction of mathematics into biology is justified by one result only—a sharper mental focus of natural phenomena.

The analysis of vegetation resolves itself into two major problems, classification of plant communities and distribution of individual species within communities. The problem of classification is essentially philosophical and a correspondingly diffuse literature has accumulated, touching on such questions as whether communities are discrete units, or whether a taxonomy of vegetation is quite extrinsic to vegetation itself. This literature has already been reviewed by Pavillard in this journal (29). Some of the points he raises must be reiterated here since they require a different emphasis in their present context. The second problem, the analysis of the distribution of species, has received little attention until recently, apart from the observations inherent in the words 'abundant,' 'local,' 'rare,' familiar to field botanists. It is the purpose of this essay to examine the application of statistical methods to the specific questions arising in these two categories of plant sociology. No attempt is made to survey the literature comprehensively and these pages can serve as little more than a prelude to the original work.

THE CLASSIFICATION OF VEGETATION

It is significant that the foundations of ecology were laid ages before the advent of ecologists. Words like 'prairie,' 'maquis,' 'moor,'

'steppe,' 'veldt' prove that the common man is capable of classifying vegetation with some precision. When these words occur in literature they summon up vivid images of the appropriate vegetation. It needs little reflection to understand that the essence of this classification is subjective, based on the physiognomy of the most prominent plants. The logic which leads a man to distinguish between a prairie and a savannah is the same logic whereby he distinguishes a dog from a jackal. In the designation of these subformations, as we might now call them, we have not progressed much farther than the founders of our language, for we find their classification adequate. Its adequacy can be illustrated by reference to the remarkable survey of African vegetation by Shantz (37), or to Livingston and Shreve's work on the climate and vegetation of the United States (23).

This subjective approach to ecology is still followed in the analysis of more local communities. The beechwoods of Europe, the mesquite of Arizona, the garigues of France, are all recognized by the practiced eye of the ecologist and assigned to their categories according to his personal judgment. The difficulties of classifying transitions were largely removed by Cowles' brilliant concept of succession (11), and although there may be disputes over individual cases, there is general agreement as to the canons of criticism to be used in plant sociology. Confusion there certainly is, but it is no worse than the parallel confusion among taxonomists over genera like *Hieracium* and *Rubus*.

As early as 1902 Jaccard (18, 19) attempted to establish more precise criteria for classifying plant communities. He compared the number of species common to two communities with the total number present in both, and expressed the result as a percentage. In the comparison of two exactly similar alpine meadows the coefficient did not exceed about 60 per cent, from which Jaccard concluded that the physiognomic criterion was deceptive, but he did not on that account relinquish the old method of classification. Moreover, the statistical method was not sufficiently sensitive, for a species which was abundant in one area and very rare in another would be classed as common to the two areas. The relative prominence of plants was left out of account.

The quadrat was introduced into ecology by Clements in 1905 (10), but it is significant that neither he nor Shantz, who used the

method extensively (36), departed from the personal estimation of communities as a basis of classification. Quadrats were used to describe the composition of communities rather than to classify them.

In 1909 Raunkiaer introduced the method of percentage frequency analysis into ecology (31). The procedure consists in throwing a quadrat repeatedly over the area to be examined and noting merely the presence or absence of the various species. Data are collected quickly and the method minimizes the personal error inevitable in the quasi-quantitative methods of Braun-Blanquet (8). From such data the community can be described in terms of the percentage frequency of its constituent species. The results are grouped in five valency classes:—0–20, 21–40, 41–60, 61–80, 81–100 per cent. It must be emphasised that Raunkiaer did not use the percentage frequency analysis as a basis of classification. He selected his units of vegetation by experience and used the quantitative data to summarize the internal composition of the community. Raunkiaer did suggest, however, that the percentage frequencies were distributed according to a 'law,' namely, that the five valency classes stood to one another in the relation $A > B > C \begin{smallmatrix} > \\ < \end{smallmatrix} D < E$, i.e., in a J-shaped distribution.

In 1918 du Rietz and his collaborators published the first of a large number of papers (32, 33, 34, 35) in which the percentage frequency method was for the first time adopted as a basis for classification. It has been mentioned above that a maximum frequency occurs in the highest valency class, i.e., several species occur in almost every quadrat thrown on the community. It is this feature of Raunkiaer's work which du Rietz extended beyond its purely descriptive function and used as the basis of classification. He defined an association¹ as a community containing certain definite constants, i.e., species which occurred in 90–100 per cent of the quadrat-throws. The number of constant species depends, of course, on the size of the quadrat, but the relationship between quadrat area and number of constants is logarithmic rather than linear, so that a quadrat area is reached above which there is no striking increase in number of constants. This area, which cannot be determined with

¹ The unit of vegetation under discussion was called by du Rietz an *association* until 1927, when he changed its name to *Soziation* (35).

any precision, was defined—by a curiously circular argument—as the smallest area on which the association attains its definite number of constants!

The method was used, as Pavillard (29) has put it, to 'pulverize the vegetation' of the Scandinavian countries. The effectiveness of this process, and the consequent bewildering number of associations, is well illustrated by the following two examples. In the beechwoods of Scandinavia Lindquist (22) distinguishes thirteen associations in which *Asperula odorata* dominates: *Asperula-Anemone*, *Asperula-Galeopsis*, *Asperula-Lamium*, *Asperula-Melica*, etc. Oswald (28), in a stretch of moorland five miles by eight, recognizes 164 associations, and many 'Assoziationsflecken.'

It is unnecessary to labor the insufficiency of this attempt to classify vegetation, for the method has been abandoned by its inventors and its shortcomings have been examined elsewhere (5, 20, 27, 39). As a guide to any future attempts to classify vegetation statistically, it may be well to summarize the results of these examinations.

1: Any statistical method rests on the assumption that samples are taken at random from the population. In the method of constants the population is not sampled randomly (35), and when it is so sampled there are often no constants at all.

2: It has been shown by Kylin (20) and others on theoretical grounds, and by the present writer from field data (5), that the density (plants per unit area) is not a direct measure of the percentage frequency, but is approximately proportional to the logarithm of the frequency (*vide infra*). The 90–100 per cent frequency class embraces a very wide range of densities, sometimes a hundred times the range of the 80–90 per cent class. The maximum in the 90–100 per cent class, which is the foundation of du Rietz' method, depends, therefore, on the technique of investigation and not on any peculiarity of the vegetation. Furthermore, the plants which occur as constants are those whose area is small in comparison with the quadrat-area. Prominent plants are often excluded, so that the constants in a beechwood may turn out to be not beech trees, but *Oxalis* or *Mercurialis*.

3: This consideration introduces the third drawback of the method, the fact that its results do not always accord with common-sense observation. The community will always be recognized by its

prominent species, even if it is characterized by some obscure moss (33, 160). Implicit in the statistical analysis of the Uppsala school is a purely subjective choice of the constants. Their classification is in fact first made in the conventional manner, and the so-called statistical analysis imposed afterwards. This procedure is perfectly sound, so long as the investigator is not deluded into supposing that his classification is objective and based on the statistical analysis.

The writer's apology for describing a failure at such length is two-fold. In the first place the work of the Uppsala school is often condemned without a clear idea of its weaknesses, and some of its critics have fallen into the same errors. Secondly, the writer wishes to advance the following conclusions. The classification of vegetation still rests upon personal judgment and intuition which cannot be replaced by any mechanical quantitative method. A statistical method even if mathematicians could provide such a method, would still have to be built upon a subjective primary classification. Efforts to set this aspect of plant sociology on a quantitative basis have merely confirmed the conventional methods employed by Cowles and his followers in America (11) and by Tansley (40), Moss (26), Watt (41) and others in England.

THE DISTRIBUTION OF SPECIES WITHIN THE COMMUNITY

Statistical methods are worthless as a basis for classification, but applied to the distribution of individual species they present remarkable possibilities. The specific problem may be stated in the following terms. If the individuals of a species are distributed randomly in a community, the sole agency which can be invoked to explain their distribution is chance. In other words, the conditions determining distribution are homogeneous over the area studied. The problems of experimental ecology arise from the fact that often the individuals of a species are not distributed at random. They are either aggregated or they give evidence of a mutual 'antagonism,' i.e., they are spaced further apart than they would be according to chance. Immediately the question is raised as to the cause of this heterogeneity. Does it lie in the microclimate, in the soil, or in the plants' relations to one another? Some technique for estimating distribution is clearly necessary. A second problem is the determination of the relative proportions of various species present in a community. The question often arises in agriculture where it is important to know

whether grazing or fertilizing a pasture leads to the increase of one species at the expense of another. Similar situations arise in academic botany, when the history of a species is followed through various stages of a succession, or when the distribution of a species is correlated with the distribution of some causal factor, such as hydrogen ion concentration. To all these problems statistical methods bring more precise definition and more certain knowledge. In the following pages the application of such methods is illustrated by reference to particular examples.

PREREQUISITES FOR STATISTICAL ANALYSIS

Before statistical methods can be employed at all, certain conditions have to be observed. Samples must be taken at random. The random sampling of a plant community is not always easy and the following two examples illustrate the precautions which are necessary. The first method consists in choosing some base line alongside the community (a path or a railway) and drawing on a map perpendiculars from this base line into the community, the perpendiculars being randomly distributed along the base line and of random lengths. Samples are then taken in the field from the ends of the perpendiculars. A second method is to draw a number of straight lines across a map of the community at random, and to walk along these by compass bearing, taking samples at regular intervals.

A second requirement is that data should be susceptible to analysis. Samples are usually collected from a quadrat (a rectangle is more trustworthy (9)). The data may be counts of individuals, tiller counts, estimates of dry weight or of area covered, or percentage frequencies. For the purposes of analysis counts of individuals are the most satisfactory. Where individuals cannot be distinguished, some morphological character, like tiller count, is suitable. If this is impracticable, estimates of percentage area covered have to be made but the data so obtained are of less value because they cannot be treated by simple statistical analysis (*vide infra*). Finally, the data are often collected as percentage frequencies. These data are still more awkward to handle. They bear no predictable relation to the actual density, unless the distribution is random, and it would be precarious to draw any conclusions as to the internal composition of a community from percentage frequencies alone.

TESTS FOR RANDOM DISTRIBUTION

Where it is possible to count individuals, the data collected from several quadrat throws may be classified into the number of throws in which no individuals occurred, the number in which one occurred, the number in which two, three, four, occurred, and so on. The quadrat size is adjusted so that the great majority of the quadrats are empty. Under these circumstances the chances of finding 0, 1, 2, 3, 4, ... x individuals per quadrat is given by the successive terms of a Poisson series:—

$$e^{-m}, me^{-m}, m^2/2! e^{-m}, m^3/3! e^{-m} \dots m^x/x! e^{-m}$$

where m is the mean frequency of occurrence in the samples, i.e., the total number of individuals counted divided by the number of quadrats. The procedure in testing for homogeneity is to compare the observed and calculated frequencies by means of the χ^2 test, in the manner described by Fisher (12). An example of the operation of this method is given in the following table:—

TABLE I

Number of individuals	Number of quadrat throws:—	
	Observed	Calculated
0	268	264.9
1	262	264.9
2	136	132.4
3	43	44.1
4	11	11.0
5	0	2.2
6	0	0.4
Total	720	719.9

The value of m is .97; e^{-m} is, therefore, .379. The column of calculated values is obtained by working out the terms of the Poisson series, and multiplying each by 720. An example of the application of this technique is to be found in Blackman's work on pastures (7). Blackman's conclusions from observations on a great number of pasture plants are that the common species are distributed at random, and the rarer species are aggregated, i.e., the distribution of dominants accords with the expectation from Poisson distribution,

while that of the occasional species does not. If it is once established that a species is randomly distributed, the number of individuals in any homogeneous area can be calculated simply from knowledge of the number of quadrat throws in which the species does *not* occur, for the zero class of the Poisson distribution, e^{-m} , determines the rest of the distribution.

Such a comparison is excellent as a test of homogeneity, but it gives no measure of the degree of non-randomness of a heterogeneous distribution. A method has recently been published which measures the degree of aggregation or of under-dispersion in a population of individuals (5). The experimental procedure involves sampling the population with a quadrat divided up into a lattice of 16 or 25 equal squares. The observations taken are (1) the total number of individuals in the quadrat, and (2) the number of empty squares. In a lattice of 25 squares, if there are no plants, there will be 25 empty squares. Above a certain density all the squares will contain at least one plant,—there will be no empty squares. Between these extremes there will be a relation between the density and the number of empty squares; this relationship is given by the equation

$$E = n \left(\frac{n-1}{n} \right)^s$$

where E is the number of empty squares, n the total number in the lattice (25 in this case) and s the density, i.e., the number of individuals in the whole quadrat. Now if individuals are clumped together, there will be more empty squares for a given density than would be expected by chance. If the individuals are mutually antagonistic the observed number of empty squares in the quadrat will be less than the calculated number. Departure from a random distribution is tested by comparing the difference:

Sum of $E_{\text{cal.}}$ and Sum of E_{observed}

with the variance of the observations. If departure from randomness is significant it can be estimated by applying the following correction to the equation:

$$E_{\text{corrected}} = n \left(\frac{n-1}{n} \right)^s \left\{ 1 + s(s-1)c \right\}$$

The value of c is a measure of the heterogeneity, i.e., it measures the degree of aggregation among clumped species or the degree of

antagonism between dispersed species. For an example of the operation of this method the reader is referred to the original work (5). The data obtained are useful in that they indicate where the problem lies. Aggregation is often found, for instance, where there is no obvious reason why the individuals should be clumped together; and in cases where competition occurs between individuals, the degree of competition may be measured.

Very recently (9a), Clapham has published a statistical analysis of some data of Steiger (Ecology, 11, 1930) on the distribution of prairie plants. He estimates the degree of departure from a Poisson distribution, i.e., the degree of non-randomness, by the relative variance:—

$$\frac{(x - m)^2}{m(n - 1)}$$

where $(x - m)$ is the deviation of an observation x from the mean, m , and n is the number of observations. This expression is summed over the observations. In a random distribution the relative variance is equal to unity, and it is greater than unity if there is aggregation. Its magnitude is a measure of the amount of aggregation. This method has an advantage over that suggested by the present writer (5), namely, that it is more convenient to use and less laborious to work out. As yet, no comparison has been made of the estimates given by the two methods. Using his method Clapham shows that most of the species listed by Steiger show aggregation. He points out that as a consequence of the over-dispersion of species the percentage frequency cannot be used for estimates of density, nor is an estimate of mean density itself of much value to the ecologist.

THE MANIPULATION OF DATA ON TILLER COUNTS

If the tillers of a species are normally distributed the frequencies will lie on a binomial distribution. Blackman has plotted the distributions for various pasture grasses in England, and finds that the dominant grasses in a sward are in fact normally distributed, while the distributions of the less common grasses are markedly skew. The causes of this departure from normality have not received experimental investigation.

MEASUREMENTS OF PERCENTAGE AREA COVERED

Where the counting of individuals or tillers is not feasible, the investigator must fall back on estimates of the percentage of area

covered (Deckungsgrad). Such estimates serve well for the examination of gross changes in the composition of a community (*vide infra*) but they are unsuitable as a means of testing homogeneity. The reason is obvious. A test of homogeneity must be made on discrete units of the same size. Any statistical test of units which themselves vary in area (e.g., patches of clover in a lawn) is invalid. Both Blackman (7) and Hanson (17) have published distribution curves of percentage area covered. The curves are extremely skew, so much so that the testing of homogeneity from replicate plots by analysis of variance, as Hanson has done (17), is probably not admissible. The analysis of variance is becoming increasingly useful as an instrument for the analysis of biological data. It is important to remember, therefore, that its value decreases as the distributions to which it is applied depart further from normality. To this particular instance of area distributions, as Blackman has pointed out, the analysis cannot be legitimately applied unless some sort of transformation is carried out on the original data, to restore them to normality.

PERCENTAGE FREQUENCY DATA

Observations on species distribution which consist solely of percentage frequency measurements can provide no information as to homogeneity. At a previous point in this essay the inherent ambiguity of the method was mentioned. This ambiguity lies in the non-linear relation between density and percentage frequency. The relation in a homogeneous population is given by the equation

$$P = 1 - e^{-kx}$$

where P is the frequency, x the density, and k the size of the quadrat. Now, although there is no direct proportionality between P and x over their whole ranges, values of P are approximately proportional to x over a narrow range of frequencies. By an adjustment of the quadrat size so that most of the densities fall into the 80 per cent frequency class or lower, and none in the 100 per cent class, the percentage frequency becomes an approximate measure of the density. A mathematical demonstration of this principle is to be found in the appendix to Blackman's paper (7; 775). It should be emphasized that as an estimate of density this technique is a last resort, for it depends on the assumption of homogeneity, an assumption which is often unjustified.

THE USE OF STATISTICS IN FOLLOWING CHANGES IN RELATIVE
COMPOSITION

The importance of being able to follow changes in the relative composition of pastures has led to several statistical methods of pasture analysis, which differ rather in their experimental technique than in their logical basis. The percentage frequency method may be set aside as useless, except in the modified form suggested above. For example, if measurements are made with a 100 cm² quadrat, a change from 2 plants per quadrat to 16 per quadrat would involve *no* change in percentage frequency, while a change from 2 plants per quadrat to one plant would be recorded as a change of 50 per cent!

Changes in botanical composition may be followed easily by tiller counts, or measurements of percentage area covered. The technique applied to the analysis of manurial effects on lawns is well illustrated by the following table from Blackman (7); the figures represent percentage areas covered by three constituents; two grasses and the total "weeds," under two treatments and the control.

TABLE II
PERCENTAGE AREA COVERED

	Control		Sulphate of ammonia		Ferrous amm. sulphate	
	June	October	June	October	June	October
<i>Poa</i> spp.	13.3	22.0	34.1	38.0	38.3	58.8
<i>Agrostis</i> spp.	31.5	36.9	53.2	58.0	48.4	41.4
Total "weeds" ...	36.2	33.8	11.7	5.0	12.5	0.3

The differential effect of fertilizers in this instance is evident without further analysis. The same technique has been applied by the writer to an examination of the change in abundance of *Galium saxatile* and other species at the transition between one association and another, on the slope of a mountain in Wales. It is evident from the results that the transition between the two associations is abrupt, not only for the dominant species, but for many of the subsidiary species too. The cause of this abruptness lies probably in the influence of the dominant species upon the local environment of the

less pretentious plants in the association, and it cannot be invoked as evidence of the individuality of associations (*cf.* 33).

In Australia changes in relative composition have been followed by Levy and others (21) by the "point" method. Hanson (17) has compared this with other methods of collecting data on botanical composition and finds a general agreement in results. The mode of dealing with the data is the same.

THE CORRELATIONS OF SPECIES DISTRIBUTION WITH ENVIRONMENTAL FACTORS

Statistical methods are appropriate for investigating the causal relation between plant distribution and some environmental factor. In such an enquiry it is necessary first to unravel the distribution of the species in question from the distribution of the factor under observation, and subsequently to seek some correlation between them. It has been alleged, for instance, that there are in Nature definite pH optima for some species. It is easy to understand how this opinion arose. *Pteris*, for instance, is found in England on soils of pH 5.6 more commonly than at any other pH. Statistical analysis showed, however, that the cause of this is *not* a peculiar preference of *Pteris* for that particular pH, but is merely the circumstance that a pH of 5.6 is more frequent than any other pH on these particular sandy soils. The distribution of telegraph poles on these soils would also show an optimum pH at 5.6. The method of analysis employed in this instance is extremely simple. Frequencies of *Pteris* were expressed as percentages of the frequencies of pH. These percentage occurrences of *Pteris* showed no preference for any particular pH, i.e., within the range at which *Pteris* will grow at all, one pH is as good as another. The comparison of observed and calculated percentages was made by means of the X^2 test (13).

By a similar procedure the abundance of one species may be correlated with that of another. A significant correlation may indicate competitive effects. Often two species are surprisingly independent of one another in their distribution, i.e., they seem to co-exist in the same quadrat without getting in each other's way. Thus Hanson (17) calculated the correlation between the tiller counts in the same quadrat of *Bouteloua* and *Agropyron*, and obtained a value for the correlation coefficient of $.09 \pm .034$, which is insignificant.

SPECIES AND AREA

Several attempts have been made to derive a formula for calculating the probable number of species in an area from observations of the number of species in small samples of the area. Arrhenius (1, 2, 3) suggested a formula which he illustrated by data of his own and which was subsequently applied to data of Gleason (14, 15) and du Rietz (34). Gleason showed that the formula gave results far in excess of the observed values, when applied to large areas, and du Rietz gave evidence that the agreement failed at areas as low as 4 square metres. The premise of Arrhenius' formula is unsound, for the expression is parabolic in form, and, consequently, with increasing area the number of species increases to infinity! Gleason put forward another formula which has the merit of being theoretically sound, for it is logarithmic, and hence is asymptotic along the area axis, i.e., it does not presuppose an infinite number of species. If it holds over large areas it does presuppose, however, equality of environmental conditions, and Gleason has used the successful application of his formula as evidence of such homogeneity.

The requisite procedure in order to verify Species-Area equations in general is to take readings of the number of species in quadrats of different areas laid at random over the community. It is not legitimate to 'compose' the readings for larger quadrats by combining the data from the small ones, for this treatment cancels out any inequalities in distributions. Since most of the data purporting to verify these equations have been collected in this erroneous way, it is premature to discuss the value of the equations themselves.

CONCLUSIONS

A study of the literature to which this essay is an introduction will convince the student that statistics have been applied to the most varied ecological problems, often needlessly, and sometimes on false assumptions. It is a literature remarkable for its prolixity, but from its pages there emerge methods which are new to biology and which prove valuable in the difficult study of plant distribution. It must be left to the future to decide in what measure these methods can advance botany.

BIBLIOGRAPHY

1. ARRHENIUS, O. Distribution of species over the area. Meddl. Kgl. Vet. Nobelinst. 4: 1920.
2. ———. Species and area. Jour. Ecol. 9: 95. 1921.

3. ———. Statistical investigations on the constitution of plant associations. *Ecology*. 4: 68. 1923. (The formula set forth in these papers has been examined by Gleason, and demonstrated to be unsound.)
4. ASHBY, E. Quantitative methods in the analysis of vegetation. *Proc. Linn. Soc.* 146: 30. 1933.
5. ———. The quantitative analysis of vegetation. *Ann. Bot.* 49: 779. 1935. (Contains an examination of the methods advocated by the Uppsala school, and the mathematical basis of a new method of analysing species distribution.)
6. BLACKMAN, G. E. An ecological study of closely cut turf treated with ammonium and ferrous sulphates. *Ann. Appl. Biol.* 19: 204. 1932. (Examples of the application of the percentage area method.)
7. ———. A study by statistical methods of the distribution of species in grassland associations. *Ann. Bot.* 49: 749. 1935. (Contains applications of Poisson series to distributions and a critical analysis of the various statistical methods in vogue.)
8. BRAUN-BLANQUET, J. *Pflanzensoziologie*. Leipzig. 1927.
9. CLAPHAM, A. R. The form of the observational unit in quantitative ecology. *Jour. Ecol.* 20: 192. 1932.
- 9a. ———. Over-dispersion in grassland communities and the use of statistical methods in plant ecology. *Jour. Ecol.* 24: 232. 1936.
10. CLEMENTS, F. E. *Research methods in ecology*. Lincoln. 1905. (First account of the quadrat method.)
11. COWLES, H. C. The physiographic ecology of Chicago. *Bot. Gaz.* 31: 73. 1901.
12. FISHER, R. A. *Statistical methods for research workers*. London, 1934.
13. EMMETT, H. E. G. AND ASHBY, E. Some observations on the relation between hydrogen ion concentration of the soil and plant distribution. *Ann. Bot.* 48: 869. 1934. (Demonstrates that the apparent optimum pH for a species may be due to the distribution of pH values themselves.)
14. GLEASON, H. A. The relation between species and area. *Ecology* 3: 158. 1922.
15. ———. Species and Area. *Ecology* 6: 66. 1925. (Contains criticisms of Arrhenius's formula.)
16. HANSON, C. AND BALL, W. S. An application of Raunkiaer's Law of Frequency to grazing studies. *Ecology* 9: 67. 1928.
17. HANSON, C. A comparison of methods of botanical analysis of native prairie in western North Dakota. *Jour. Agr. Res.* 19: 815. 1934.
18. JACCARD, P. Lois de distribution florale dans la zone alpine. *Bull. Soc. Vaud.* 38: 69. 1902.
19. ———. Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaud.* 44: 223. 1908. (Applications of the coefficient of community.)
20. KYLIN, H. Ueber Begriffsbildung u. Statistik in der Pflanzensoziologie. *Bot. Not.* 2: 81. 1926. (An extensive mathematical treatment of the problems of statistical ecology; a very important paper.)
21. LEVY, B. AND MADDEN, E. A. The point method of pasture analysis. *New Zealand Jour. Agr.* 46: 267. 1933.
22. LINDQUIST, B. *Den Skandinaviska Bokskogens Biologi*. Akademisk Avhandling. Stockholm, 1931.
23. LIVINGSTON, B. E. AND SHREVE, F. The distribution of vegetation in the United States in relation to climatic conditions. *Carnegie Pub.* 284. 1921.
24. MCGINNES, W. G. The relationship between frequency index and abundance as applied to plant population in a semi-arid region.

- Ecology 15: 263. 1934. (Embodies data which indicate homogeneity in the distribution of semi-desert vegetation.)
25. MORGAN, A. AND BERULDSSEN, E. T. Sampling technique as applied to irrigated pasture, etc. Jour. Dept. Agr. Victoria 29: 36. 1931.
 26. MOSS, C. E. The vegetation of the Peak District, Cambridge, England, 1913.
 27. NORDHAGEN, R. Om homogenitet, konstans, og minimareal. Nyt. mag. f. naturvid. 60: 1922. (One of the first publications exposing the fallacies of the Uppsala school.)
 28. OSVALD, H. Die Vegetation des Hochmoores Komosse. Akademische Abhandlung. Uppsala, 1923.
 29. PAVILLARD, J. The present status of the plant association. Bot. Rev. 1: 210. 1935.
 30. PEARSALE, W. H. The statistical analysis of vegetation. Jour. Ecol. 12: 135. 1924.
 31. RAUNKIAER, C. Life forms and statistical plant geography. Oxford, 1934.
 32. DU RIETZ, G. E., FRIES, T. C., OSVALD, H. AND TENGWALL, T. A. Gesetze der Konstitution natürlicher Pflanzengesellschaften. Vetensk. ock. pract. unders: i. Lappland, etc. Flora och Fauna, 7: 1920.
 33. DU RIETZ, G. E. Zur methodologischen Grundlage der modernen Pflanzensociologie. Ak. Avh. Uppsala, 1921.
 34. ———. Ueber das Wachsen der Anzahl der konstanten Arten &c. Bot. Not. 1922, p. 17.
 35. ———. Vegetationsforschung auf sozionsanalytischer Grundlage. Abderhalden's Handbuch der biologischen Arbeitsmethoden. 11: V, 293. 1930.
 36. SHANTZ, H. L. A study of the vegetation of the mesa region east of Pike's Peak. Bot. Gaz. 42: 1906.
 37. SHANTZ, H. L. AND MARBUT, C. F. The vegetation and soils of Africa. Amer. Geog. Soc. Res. Ser. 13. New York, 1923.
 38. STAPLEDON, R. G. Pasture problems: drought resistance. Jour. Agr. Sci. 5: 132. 1912-13. (An early description of the weight-productivity method of determining botanical composition.)
 39. SVEDBERG, T. Et bidrag till de statistiska metodernas användning inom växtbiologien. Svensk. Bot. Tidskrift 16: 1. 1922. (Critique of the minimal area method of analysis.)
 40. TANSLEY, A. G. Types of British vegetation, Cambridge, England, 1911. (An example of the "conventional" method of describing and classifying vegetation, which, in the author's opinion, is still the only practicable method.)
 41. WATT, A. S. On the ecology of British beechwoods. Jour. Ecol. 11: 1. 1923; 12: 145. 1924; 13: 27. 1925.
 42. WIEHE, P. O. A quantitative study of the influence of tide upon populations of *Salicornia Europea*. Jour. of Ecol. 23: 323. 1935.

THE PHYSIOLOGY OF HOST-PARASITE RELATIONS

WILLIAM BROWN

*Professor of Mycology and Plant Pathology, Imperial College of
Science and Technology, London, England*

INTRODUCTION

In reviewing the subject of parasitism from the physiological standpoint, little useful purpose would be served by confining attention to the literature of the last few years. References to the physiological relations of host and parasite are often of a casual nature, made in the course of investigations which had some other object in view, and even if one succeeded in collecting and evaluating these rather miscellaneous observations or interpretations, no satisfactory picture of the problem would emerge. It is proposed, therefore, more especially as this is the first of these reviews which deals with the physiology of parasitism, to make a general survey of the literature, in the light of which the more recent developments will be more readily understood.

The earliest substantial contribution to the subject is by De Bary (6) in a classical paper dealing with the mode of invasion of *Sclerotinia libertiana*, a non-specialized type of parasite. Examination of infected tissue (broad bean, carrot, etc.) showed that two types of action had occurred; one, the solution or partial solution of certain constituents of the cell walls whereby the tissues lost coherence or in other words were rotted; and the other, the killing of the protoplasts themselves. De Bary further demonstrated the presence, in extracts of tissues parasitized by the fungus, of an active principle which produced the effects described. Boiling of these extracts in general destroyed their activity, whence the conclusion was drawn that the effect on the cell wall was due to an enzyme. The nature of the toxic or killing substance was less clear and De Bary, in effect, left that question open. In this connection he considered oxalic acid, which is known to be produced by a number of fungi, but the evidence that this acid was produced in sufficient concentration by *Sclerotinia libertiana* was unsatisfactory.

In studying the earliest phase of attack, i.e., penetration of the intact surface of the host, De Bary reached certain conclusions which

have markedly influenced subsequent workers in this field. By a variety of experiments he showed that the hyphae of *Sclerotinia*, when placed on a suitable plant part, attacked only after they had been given a certain amount of feeding, whence his conclusion that parasites of this type achieve parasitism by way of an intermediate stage of saprophytism. When the hyphae were placed in a nutrient medium on the intact surface of a susceptible host, De Bary claimed that they penetrated directly, whereas when placed in water they were unable to do so at first. Instead, they applied themselves to the host surface and formed attachment organs ("appressoria"). From these a toxic principle was excreted into the underlying living cells which were thereby killed. The soluble contents of the dead cells now diffused out to the surface where they supplied the saprophytic feeding necessary for the establishment of parasitism.

It is important to appreciate this point because, if true, it would indicate a possible difference between a parasitic and a saprophytic fungus, the former having the capacity of killing the underlying host cells before penetration had taken place, and the latter presumably being unable to do so. Incidentally, it may be noted that De Bary was unable to explain satisfactorily this "killing in advance of penetration" on the basis of any of the extracts prepared by him. When these were laid on the epidermis of a plant, action was very uncertain and in many cases there was none. He suggested, rather weakly, that the killing principle was deactivated before it had time to diffuse through the cuticle.

While, as will be shown later, there are grounds for not accepting the thesis of killing in advance of penetration, there is general agreement that, once a fungus of the type under consideration has entered the tissues of its host, there is a more or less well defined killing and maceration of the host cells in advance of the region occupied by the hyphae.

De Bary's work was in the main confirmed and in certain respects amplified by Marshall Ward's (138) study of a lily disease caused by a species of *Botrytis*, and by Nordhausen (106) who examined the behaviour of a number of fungi, more particularly that of *Botrytis cinerea*, a widely distributed parasite. Similar conclusions were reached by some of the earlier students of bacterial plant diseases, e.g., by Jones (81) and Van Hall (135) for strains of *Bacillus carotovorus*. On the other hand, Behrens (7) claimed that the toxic

principle of certain fruit-rotting fungi was a thermostable substance, and an extreme position was taken up by Smith (124) when he stated that both the killing and macerating effects of *Botrytis cinerea* on lettuce tissue are brought about by oxalic acid secreted by the fungus.

A study of the earlier papers cited above shows that in no case was the fungal extract employed of reasonable activity. The experimental method was to immerse pieces of sensitive tissue (turnip, potato, etc.) in the extract and to note the changes brought about. Immersion in itself is injurious to the tissue if it is long continued, and to that extent the action of any active principle concerned is obscured. To judge from the records of earlier experiments, the reaction times were unduly long—often from one to several days. In so far as these experiments had reference to a toxic principle, an antiseptic could not be added to the preparations, as is usual in enzymic work, so that there was the further difficulty arising from bacterial contamination. Again, some at least of the preparations used were obtained from old cultures of the organism, e.g., in one of Behrens' experiments from a three-month-old culture of *Penicillium* sp. on apple fruit, so that there is the double uncertainty as to how far the active principle of the fungus was contaminated with host residues and with fungal products which are associated with old and degenerate mycelium.

With these considerations in mind, Brown (14) prepared an extract from the young germ tubes of *Botrytis cinerea* and, as this proved to be of high activity, it was very suitable for the study in question. This extract actively decomposed a great variety of tissues, such as vegetable and fruit tissues generally, floral parts, the leaves of many plants and the stems or leaves of succulents. Lignified parts were, as far as could be seen, unaffected and so also were the delicate tissues of mosses and liverworts. The action was of the twofold nature outlined by De Bary. Heating for a short time to 60–70° C. destroyed the activity of the extract as regards both killing and macerating effects. Attempts were made in various ways—by deactivation of the enzyme by heat, by mechanical agitation, by the addition of a suitable small quantity of alkali, by precipitation with alcohol, and by fractional dialysis with a series of graded membranes—to separate an enzymic from a toxic principle but with no success. Though, as was pointed out

at the time (20), none of these experiments was definitely critical on the point, the obvious interpretation of the results was that both activities concerned were due to one and the same substance, a cytolytic enzyme ("pectinase," in the older literature "cytase"). Certainly there was no thermostable crystalloidal toxin present in the extract of *Botrytis* germ tubes, and oxalic acid in particular, as the killing agent, was further excluded by the fact that the extract contained a trace of a soluble calcium salt.

Similar active preparations, though not so pure as the one just described, were obtained (16) by using the liquid in which a dense suspension of *Botrytis* spores had germinated. The active principle is thus freely excreted into the nutrient medium. Under a given set of cultural conditions, it reaches a maximum concentration in the medium, but being somewhat unstable in solution it gradually disappears.

While the preparations so obtained fully explained the action of *Botrytis cinerea* on susceptible tissues, they did not explain the initial entry of the parasite (15). Even the strongest extracts were, with rare exceptions, quite innocuous when laid on the intact surface of susceptible plant parts. The cuticle was not attacked and the underlying tissue was not disorganized. The conclusion, therefore, was either that an important principle had not been extracted or that the "killing in advance of penetration" claimed by De Bary was unfounded, at least for *Botrytis cinerea*. Cytological investigation was taken up at this point when it was shown by Blackman and Welsford (9) for *Botrytis cinerea*, by Boyle (11) for *Sclerotinia libertiana* and by Dey (33) for *Colletotrichum lindemuthianum* that the process of penetration preceded killing of the underlying host cells. Once the cuticular barrier had been passed, killing and macerating effects quickly followed. By use of an electrical conductivity method, Brown (17) also showed that the rapid exosmosis of solutes from the tissues does not take place until some of the fungal hyphae have penetrated. The effect of these investigations was, therefore, to cast doubt upon De Bary's view according to which parasitic fungi of the facultative type are able to attack the cells of the host plant prior to penetration of the cuticle.

THE STAGES OF PARASITISM

In the light of the general picture outlined above, a review of the literature bearing on the physiology of parasitism will now be set

out, and it will aid to a methodical treatment if the subject is subdivided according to the stages which can be distinguished in the process of establishing parasitism, viz., the stage before penetration, penetration itself, and the stage after penetration. The discussion under the first two headings will be general, as there is no evidence that the behavior of one type of fungus is essentially different from that of another. When the third stage is reached, a further subdivision will be necessary as important differences then arise as between facultative and obligate parasites. Throughout the discussion it will be assumed that the parasite concerned is a fungus; with slight modifications here and there the same considerations apply to bacterial parasites.

Stage before Penetration. The factors which come up for discussion are those which influence the germination and growth of the parasite. Fungal spores have a capacity for germinating in pure water which depends upon the species, the age and pre-treatment of the spores. Some are able to germinate vigorously on their own reserves and are to that extent more capable of parasitising than others which require an extraneous source of food for the purpose, i.e., the "saprophytic nourishment" postulated by De Bary. Under natural conditions suitable for infection, the spores are, however, not always, or perhaps even often, in contact with pure water on the surface of a plant. By laying drops of distilled water on the intact surface of a variety of plant parts, Brown (17) showed that a passive exosmosis of electrolytes took place from the tissue into the drops, and that in many cases the solutes which so diffused out markedly stimulated the germination of spores. With other tissues the effect was the opposite, i.e., there was more germination in distilled water than in drops which had lain for some time on the surface of the plants. It was further shown (18) that volatile substances given off by plant tissue had a like effect upon germination, so that under conditions where these substances could accumulate in the atmosphere the parasitic vigor of any fungal spores present would be influenced.

The effect of an extraneous source of food on the initiation of disease may be illustrated by a few examples. Attack on foliage leaves may result from their contact with moribund floral parts. This is a fact well known to tulip growers. Similarly, the *Botrytis* disease of lettuce seedlings (1) generally begins by the attack of the

withered cotyledons or lower leaves. The spur-blight disease of certain fruit trees (147) has its beginning in the germination of the fungal spores on the nutritive stigmatic surface of the flowers. The dependence of the sooty-mold type of disease upon the presence of honeydew illustrates the same point, though in this case true parasitism, in the sense of invasion of the host tissue, does not as a rule occur.

The pathogenicity of soil-borne fungi is influenced in a similar way. Thus it is a very general experience that sterilization of a soil by heat for the control of a soil-borne disease is liable to accentuate the trouble, unless the operation is carried out so effectively as to eliminate the parasite completely. Similarly, workers on the foot-rot diseases of cereals all agree in stating that infection takes place with much greater readiness in sterilized than in unsterilized soils. The enrichment of the soil solution which follows from the heating process would partly explain these results but there is also considerable evidence that microorganisms in competition interfere with each other's activities, partly, no doubt, by using up the available food and partly by a deleterious action of their metabolic products. The literature of this so-called "biological antagonism," in so far as it concerns the cereal foot-rot fungi, has been recently reviewed by Garrett (47).

In addition to nourishment, two other factors which determine the behavior of the fungus in the stage preceding penetration are temperature and moisture. Plant pathological literature contains a vast number of references to the working of these factors.¹ For the present review it is sufficient to state that, for the establishment of parasitism, temperature must not range beyond certain limits which are more or less definite for each disease, and that moisture must be present, either as free water, or as water vapor at a concentration not too far removed from the saturation point. Sufficiently humid conditions must also be maintained for a time long enough to enable the spores to germinate and the germ-tubes to be established within the host tissue, whereby the parasite becomes less liable to check by desiccation. Hence the importance of continued rainy or "muggy" weather (as with *Phytophthora infestans* on potato) and of long dew periods (as with rusts and mildews).

¹ No attempt will be made in this article to review the very extensive literature which deals with the effect of environment upon the incidence of disease.

The conditions of wetness necessary for the initiation of attack may be difficult to attain on account of certain structural arrangements of the plant. Thus the surface of some plants is covered with a waxy bloom, which is more pronounced in some environments than in others, or with a dense layer of hairs, so that the surface is very difficult to wet. Drops of water which may contain the spores of a parasite readily fall off, and so the mechanism of parasitism breaks down at the very beginning. It is customary to speak of such cases under the heading of "disease escape" inasmuch as the plants may be readily attacked if the fungus is only allowed to establish contact. This distinction seems hardly logical, for such plants have in fact a structural device which defeats the potential parasite at the outset, and their resistance is no less real in that it came into play at the beginning rather than later on.

Phase of Penetration. Three ways of entry are possible; directly through the epidermis, through stomata or lenticels or water pores, or through wounds. The last may arise in the normal course of the plant's growth, e.g., at leaf-scars or at the points of emergence of lateral roots, or they may be caused by external agents, such as wind, insects, etc. Whatever mode of entry is adopted, the question arises as to whether the fungus enters by accident or whether it is attracted by some stimulus on the part of the host. When entrance takes place through the epidermis there is the further question as to how the resistance of the epidermal wall and, in particular, of the outermost cuticularized layer is overcome.

The view that the stimulus which leads to penetration is of a chemical nature was the natural outcome of Pfeffer's (109) researches on the locomotory directive movements of motile cells (zoospores, antherozoids) and was developed by Miyoshi (98, 99) working in Pfeffer's laboratory. According to this view, various substances such as sugars and salts diffuse out from the living cells of the plant and through the outer epidermal wall. Fungal hyphae which may be present on the surface react to this unilateral stimulus by turning in such a way as to grow up the gradient of attracting substance or, in other words, towards the source of the latter which is the host tissue.

Miyoshi's experimental methods, which have been largely followed by later workers, were briefly as follows. A solution of the substance under test was placed in a glass capillary which was then

laid in a drop of water containing germinating spores. More frequently the solution, solidified with agar or gelatine, was placed on one side of a permeable membrane (collodion, natural epidermis, etc.) or of a plate of impermeable material, such as mica, which was provided with a number of small holes. The spores were placed in a film of plain agar or gelatine on the opposite side. Conclusions were drawn according as the germ tubes grew towards or away from the source of the chemical substance or behaved neutrally. From experiments of the kind indicated Miyoshi constructed an elaborate theory of chemotropic attraction by certain substances and by plant extracts. Some substances were attractive, others not, and the concentration of an attractive substance must, on the one hand, exceed a certain threshold value, otherwise there would be no response, and, on the other hand, must not exceed a certain maximum, otherwise the effect would be one of repulsion. Penetration through membranes did or did not take place according as a suitable concentration of an attractive substance was or was not present on the opposite side. There are some difficulties and inconsistencies on certain points in Miyoshi's papers, but on the whole the evidence is both circumstantial and convincing.

Unfortunately for the theory of chemotropism, Miyoshi's conclusions were to a very large extent called in question by the later work of Fulton (46). Working by the same methods and with very much the same kind of fungi, he failed to obtain any such marked tropisms as had been recorded, the fungal hyphae showing on the whole as much turning towards pure water as to a solution of the presumably attractive substance. On the contrary, Fulton claimed that the only marked tropism shown was a turning away from the metabolic products produced by the fungus itself, and he, therefore, substituted for the positive chemotropism of Miyoshi a negative tropism of the kind stated. A similar conclusion, though founded on less extensive experimental work, had been reached a few years previously by Clark (26).

The subject was again taken up by Graves (58) who used the mica-plate method and adopted a hard-and-fast system in the ascertainment of his data whereby any liability to personal bias would be eliminated. His general conclusion was that there was justification for both Miyoshi's and Fulton's views, but that the negative chemotropism of the latter was the greater effect. Graves'

paper is the last of any importance on the subject, and though certain points are still rather obscure it must be accepted as the best account available for the time being.

Granted now that the two kinds of chemotropism are well established, it remains to consider how far the one or the other can be applied to explain the entrance of a fungus into its host. On this point very diverse views have been expressed. At the one extreme, Massee (94) founds a theory of parasitism upon the lines of Miyoshi's work. Susceptible plants are such by virtue of possessing in their cells certain attractive substances, and conversely resistant plants are devoid of these. A specialized parasite is one which responds to a particular substance only, whereas a generalized parasite responds to many substances. These views are very far from the truth if only for the reason that fungal hyphae have repeatedly been observed to enter the tissues of plants which they are unable to parasitize. At the other extreme, Brown (21) has argued that neither of the tropisms referred to plays any significant part in the process of penetration.

As far as the entrance of germ tubes through stomata is concerned, none of the experimental evidence alluded to is relevant. The perforated mica-plate, which has been so much used in work on chemotropism, is presumed to be a working model of an epidermis with its stomata. But there is an important difference, viz., that, whereas there is diffusive continuity through the holes of the mica-plate so that water-soluble substances can pass from one side to the other, the substomatal cavity is in general filled with air. If, therefore, there is any tropism to substances coming out through stomata, these substances must be volatile, and none of the investigations cited has furnished evidence of a positive tropism to gases. The only work which is significant on this point is that of Balls (4) who states that germ tubes of rust spores grow through holes in a rubber membrane when an atmosphere saturated with water vapor is maintained on the opposite side. Hence a tropism towards water vapor is suggested.

According to Arens (3), the zoospores of *Plasmopora viticola* tend to congregate around stomata, especially when these are open, and they behave in this way towards the stomata of many plants other than vine. The stimulus in this case cannot be water-vapor since the zoospores are swimming in water.

In considering the penetration of stomata by germ-tubes one probably should distinguish two phases, first the growth of the germ-tube up to the stoma, and second the entrance of the germ-tube into the substomatal chamber. It does not seem necessary to postulate any directive stimulus for the first of these stages, for no difficulty is involved in assuming that a germ-tube grows accidentally over one stoma or another. The second stage of the process would hardly be accidental, at least with the germ-tubes of rust summer-spores. When these arrive above a stoma they very characteristically form a swelling, thus indicating some influence emanating from the stoma, and subsequent growth is directly downwards into the sub-stomatal chamber.

Coming now to penetration through the epidermis, one might suggest that the negative chemotropism of Fulton and Graves causes the germ-tubes to grow away from a region of high concentration of fungal products, viz., the water drop in which the spores have germinated, into a region of low concentration, viz., the underlying host cells. But this argument is fallacious. The germ-tubes can respond only to differences of concentration within the infection drop, and it is more rational to suggest that the highest concentration of metabolites would arise on the sides of the germ tubes next to the epidermis, where free diffusion is hampered by the proximity of the cuticle. The result would, therefore, be that the germ-tubes would turn away from the surface of the plant.

The positive tropism of Miyoshi and Graves is, however, not ruled out so simply. It is quite conceivable that germ-tubes growing close to the epidermis may react to a one-sided stimulation of substances diffusing outwards from the tissue. On the other hand, Brown and Harvey's (19) work with spores of *Botrytis cinerea* and epidermis of *Allium cepa* showed that penetration took place freely after the strips of epidermis had been subjected to prolonged washing—which should have removed all traces of diffusible substances—and, furthermore, that the hyphae grew with equal freedom from the inner surface to the outer, i.e., with or against a possible gradient of chemotropic substance. In experiments with membranes of formalized gelatine the hyphae penetrated independently of the initial distribution of the food substance. Finally the fact that membranes of paraffin wax were readily penetrated (as had also been shown by Miyoshi) proved that a chemotropic stimulus is not necessary for penetration.

The lack of evidence in favor of chemotropic theories drives one back upon a theory which has been suggested from time to time, viz., that the stimulus to penetration is a contact one (haptotropic). While this theory has the disadvantage of not being easily explored experimentally, there are no fundamental objections to it, and all the evidence available is in its favor. The germ-tubes of fungi do react to contact, as is well shown with *Botrytis cinerea*. Contact with a hard substance induces a number of changes in the germ-tubes: the tips become rounded and somewhat swollen, growth in length is temporarily stopped, and the enlarged apex becomes firmly attached to the solid object, forming an "attachment organ." From the area of contact and at a short distance behind the extreme tip of the hypha, a fine penetration tube is put out through the underlying surface, if this should happen to be penetrable. The same sequence of appearances is seen when the spores are germinating on the surface of glass, except that of course no penetration is possible. One may, therefore, plausibly suggest that the same stimulus is operating in both cases, viz., the contact with a hard surface. Whether the physical character of the hard surface, as for example its roughness, influences in any way the nature of the response called forth is quite unknown.

With fungi which penetrate directly through the epidermis of the host the further problem arises of the mechanism of this process. Here again two theories have been advanced, a chemical and a mechanical. The general tendency has been to postulate a chemical action upon the cuticle, but the positive evidence in favor of this is not strong. As has been pointed out, Brown failed to discover any evidence of a cuticle-dissolving enzyme in the most active extracts of *Botrytis cinerea*. Thus an extract which on injection completely destroyed rose petals within a quarter of an hour produced no effect whatever when laid on the surface of petals for 24 hours, whereas spores so placed had disorganized the tissue in some 8 to 12 hours. The parallel cytological investigations agreed in indicating no solvent effect of the fungus upon the cuticle.

A somewhat different view is expressed by Wiltshire (146) in his study of the apple-scab organism. He considered that a cuticle-dissolving enzyme was present inasmuch as the cuticle became thinner when it was sloughed off by the development of the fungus within the tissue. But this does not mean that the outermost

cuticular layer, which is presumably most heavily impregnated with wax-like substances and which is the only layer significant to the process of penetration, is affected. Perhaps one of the most categorical claims in this connection is that contained in a recent paper by Chaudhuri (23). According to this paper the cuticle of *Citrus* sp. is attacked by an exudation from spores of the "wither-tip" fungus with the result that it is made easily penetrable by the fungal hyphae. The test applied is the capacity of the cuticle to take up a certain dye, but this in the writer's opinion gives no grounds whatever for drawing any conclusions relative to the penetrability of the cuticle by fungi.

The ability to penetrate membranes in a purely mechanical manner has been definitely proved for some fungi. Miyoshi demonstrated the penetration of collodion and gold-leaf films, and the writer has used membranes of paraffin wax for the same purpose. With a series of formalized gelatine membranes of graded hardness it was found that the germ-tubes of a particular fungus penetrated membranes up to a certain grade of hardness, and that beyond this point long-continued exposure did not lead to penetration. Chemical solution of the membranes is thus ruled out and the mechanical theory is the obvious one. Similarly in experiments on the penetration of *Eucharis* epidermis, Brown and Harvey (19) found that penetration took place if the leaf tissue was plasmolyzed so that the hydrostatic backing to the cuticle was removed, whereas the epidermis of a turgid leaf was not penetrated. This result can readily be interpreted on the mechanical theory but it is very difficult to understand why, if the fungus produces a cuticle-dissolving enzyme when growing on the flaccid epidermis, it should not be able to do so when the epidermis is rigid.

A necessary condition for mechanical penetration is that the fungus should be anchored to the epidermis of the host, i.e., there must be an appressorium. The thrust on the epidermis is reduced to a minimum by the fact that the penetration hypha is very slender, but even so it is inconceivable that the reaction to this thrust can be taken up by the germ-tube unless the latter is fixed to the epidermis. In this connection it might be pointed out that some of the figures which have been put forward as illustrating mechanical penetration can have no such meaning. Germ-tubes are figured as pointing down into depressions of the epidermal wall, and these

depressions have been cited as furnishing proof of mechanical pressure by the fungus. Obviously they are artifacts due to distortion of the material in preparation. It is to be remembered that the thrust is exerted and reaction to the thrust is taken up within the minute area common to appressorium and epidermis, and, therefore, any visual demonstration of pressure exerted is hardly to be expected.

The importance of the outer layer of the plant's body, whether cuticularized epidermis or cork, in preventing the entrance of parasitic fungi is well recognized. Thus there are many organisms ("wound parasites" such as *Stereum purpureum*, *Nectria galligena*, etc.) which strongly parasitize certain plants, if introduced into the tissues. The undamaged plant is, however, immune to attack. Again the strengthening of the cuticularized epidermis which takes place with advance to maturity often leads to increased resistance. For example, the attack of apple leaves by the scab or the rust fungus is much more vigorous with young than with older leaves, though in this case it is not quite certain that a progressive increase of internal resistance has not also taken place. Similarly the greater susceptibility of etiolated and "forced" plants as compared with ones grown normally is due, in part at least, to the poorer development of their protective layers. Of specific references to the subject of epidermal resistance, the following will serve. Melander and Craigie (96) have shown that the resistance to penetration, as measured by a mechanical device, of the epidermis of certain *Berberis* spp. is correlated with resistance of the plants to the basidiospores of *Puccinia graminis*. Curtis (31) and Valteau (132) have found that the resistance of plum varieties to the brown-rot fungus is correlated with thickness of the skin. According to Graf-Marín (57), the increased resistance of older barley plants to mildew attack is due to increased thickness of the cuticle. If the latter is shaved off the plants are readily infected.

The behavior of the stomata in the matter of the times of their opening and closing is claimed by some observers to determine the resistance or susceptibility of plants to certain parasites. As night, with its deposits of dew, is the period when fungal spores are liable to germinate, it is understandable that plants with stomata which remain closed until well after sunrise may escape infection, possibly because the closed stoma prevents entrance or because no

chemotropic influence is forthcoming. The evidence, however, is somewhat conflicting. Such "functional resistance" has been described for certain wheat varieties against invasion by *Puccinia graminis tritici* (Hart, 62) and earlier by Pool and McKay (111) for mature beet leaves against *Cercospora beticola*. According to Hull (71), susceptibility of maize varieties to *Puccinia sorghi* runs parallel with the number of stomata per unit area of the upper surface and a somewhat similar, though incomplete, parallelism has been indicated for the response of rice varieties to *Piricularia oryzae* (Nagai and Imamura, 100). On the other hand, little or no correlation between stomatal features and resistance is reported by Ward (138) for species of *Bromus* and *Puccinia bromina*, by Goulden and his co-workers (56) for wheat and *P. graminis*, by Radulescu (112) for wheat and *P. glumarum* and by Lepik (89) for the vine and *Plasmopara viticola*. The issue has been further complicated by the recent statement of Caldwell and Stone (22) that the germ-tube of *Puccinia triticina*, on coming over a stoma and producing the so-called "appressorial vesicle," causes the stoma to shut. The germ-tube is, nevertheless, able to enter the closed stoma. The whole subject of functional resistance is thus still rather obscure.

After Penetration. The study of the interaction of host and parasite, once the latter has entered, is of great fundamental interest from the complexity and variety of the effects shown. At this point it will be convenient to treat the facultative and the obligate types of parasite separately, for though the distinction between them is not hard-and-fast and though they possess certain features in common, nevertheless there are important dissimilarities which stand in the way of a uniform treatment. This applies particularly to the experimental methods which are available in the study of the respective types, as will appear shortly.

THE FACULTATIVE PARASITE

A sketch of the mechanism of this type of parasite has already been given and it now remains to amplify that account and to discuss the mechanisms employed by the plant in resisting fungal attack.

Briefly stated, the facultative type of parasite, illustrated by such a typical example as *Botrytis cinerea*, invades the tissues of the host by excreting a destructive principle which kills the cells and more

or less dissolves the cell walls in advance of the position of the hyphae. The fungus is thus living all along as a saprophyte, and in accordance with this feature one finds that such fungi can be cultivated, with greater or less ease, on artificial media. One is, therefore, in a position to study their metabolism under strictly controlled conditions, at least up to the limits set by chemical technique. Again, since the fungus in process of attack is living on dead parts of the plant, the physiology of its parasitism is the sum total of the physiology of its nutrition and of the mechanism whereby the living tissue is broken down by the fungal excretions. The problem thus presents no insuperable difficulties, and it is not surprising, therefore, that this type of parasitism has been the most fully explored.

The offensive mechanism possessed by the parasite consists of the products which it is able to excrete and it would be unsafe to disregard any one of these in this connection. Such products include enzymes, especially those which attack cell wall constituents, organic acids, alcohols, and many more. Chief attention has been paid to enzymes and organic acids, as has already been indicated, and varying importance has been attached by one author or another to each. Thus the writer claims that the enzymic system, in particular the pectinase, of *Botrytis cinerea* produces on the host tissue all the effects of which the fungus itself is capable. Nevertheless, it would be rash to claim that what is true of one fungus is true of others, so that there is no necessary opposition between the writer's work on *Botrytis cinerea* and that of Clayton (27) on *Bacterium tabacum*, and of Pierstorff (110) on *Bacillus amylovorus*, of Higgins (67) on *Sclerotium rolfsii* and of Johann, Holbert and Dickson (74) on *Penicillium oxalicum*. Clayton and Pierstorff ascribe the toxic action to an unspecified thermostable substance, Higgins and Johann *et al.* to oxalic acid.

In work of this description it is important to remember that the metabolic products of an organism may be widely different on different media. Hence an experimental difficulty arises in making a sufficiently close approximation to the medium in which the parasitic fungus is actually growing, viz., the tissue of the host which has been killed by the fungus. Clayton's claim for a thermostable toxin will be more readily accepted when he has shown that it is excreted by the organism as grown on tobacco leaf tissue, and, as the toxin

is apparently a relatively simple substance, when he has proceeded some distance in its identification. The claims in favor of oxalic acid are, in the writer's opinion, open to serious criticism. A careful study of the two papers cited does not give any clear proof that a toxic concentration of oxalic acid was present in the region where the fungus was invading. That a large amount of oxalic acid is produced by such and such a fungus on a particular medium is not relevant evidence, since the formation of this acid is notoriously dependent upon the composition of the medium and other cultural conditions. Also the occurrence of crystals of calcium oxalate in the parasitized tissue, to which reference is made by some workers, does not in itself show that a toxic concentration of oxalic acid is present, but rather that the amount of free oxalic acid has been kept at the vanishing point until all the soluble calcium salt has been precipitated.

The general criticism offered in the foregoing paragraph applies also to the writer's work on the enzyme of *Botrytis cinerea*, but this work has at least the advantage that certain *à priori* considerations are in its favor. In any disease where there is definite rotting of tissue the existence of a cell-wall dissolving enzyme must be postulated, since there is no plausible alternative. Extracts of very high activity have been obtained by special methods, but there is no need to postulate such a high concentration of enzyme within the tissue, since the action of the fungus is much slower than that of the extracts. The enzyme being a catalyst, a weak concentration will produce the same effect as a stronger if given sufficient time, whereas with oxalic acid the toxic effect is determined largely by the concentration.

In addition to the quantitative requirements just indicated, qualitative effects must be borne in mind. The solution of the active principle should reproduce the action of the fungus in detail, or if not, the divergence should be explainable. Judged by this criterion, some of the work on oxalic acid fails. Thus Smith (124) claimed that the toxic and macerating effects of *Botrytis cinerea* are due to oxalic acid. Apart from a complete lack of evidence that oxalic acid was produced by the fungus, there was the striking point that the acid bleached the killed tissue whereas the fungus and the fungal extracts turned it into a reddish brown. Smith's explanation of this difference is untenable. The reddish-brown

coloration is the natural post-mortem change in lettuce tissue, whenever the conditions of killing have been such as not to destroy the oxidase coloring system of the plant. The fact that oxalic acid interferes with this reaction, whereas the fungus does not, is sound evidence that oxalic acid is not the active constituent of the fungal apparatus.

The writer does not favor the theory that oxalic or any other organic acid is of prime importance in connection with fungal invasion, though it may be that in some cases an acid reaction markedly favors the progress of attack, as for example with *Penicillium* spp. on orange fruit (Green, 59). It is noteworthy that the fungus *Aspergillus niger*, forms of which are able to produce large quantities of oxalic acid, is not remarkable for its parasitic tendencies. Furthermore, many plant diseases are characterized by the development of an alkaline reaction, so that there can be no question with these of the participation of an acid. Finally, any theory based on the toxicity of organic acids would seem to be quite inadequate when one remembers the inverse relationships so abundantly illustrated in pathology. Fungus α attacks host A but not host B ; fungus β attacks B but not A . How can this be explained on the acid theory? The conditions governing acid formation in culture are fairly well known, and one of these is the presence of a large amount of readily oxidizable carbohydrate. Judged by the behavior of fungus α , the tissue of A is more favorable to acid production than is the tissue of B ; while the converse is true if one considers fungus β . The acid theory is too simple, and on that account unpromising.

The enzymic theory is not free from considerable difficulties. Thus Harter and Weimer (63) found no correlation between the parasitic vigor of some species of *Rhizopus* and their capacity to excrete pectinase on certain standard media, a conclusion which was confirmed by Paul (107) for a number of strains of *Fusarium fructigenum*. The enzyme is also known to be secreted by a number of saprophytic fungi and bacteria. These difficulties have been largely cleared away by recent work in the writer's laboratory, where a systematic study of the factors governing the secretion and functioning of the enzyme is in progress. A review of this work has been given elsewhere (21) so that a summary will suffice here. It has been found that the composition of the cultural medium affects the amount of enzyme secreted, so much so that in media with

a high preponderance of carbohydrate over the nitrogenous constituent very little enzyme is produced though there is considerable mycelial development. Conversely, in media with low carbohydrate and high nitrogen content growth may be poor but enzyme secretion is active. Furthermore, the detailed properties of the enzyme, such as sensitiveness to H-ion concentration and to various retarding chemical agents, vary from one fungus to another and even for the same fungus according to circumstances (25, 97). There is clear evidence that the properties of the enzyme are determined to some extent by the presence of some other substances. In the light of these results it is clear that no correlation between the amount of enzyme formed on any selected medium and the parasitic vigor of the fungus need be expected. A further point of considerable significance is that a strong solution of pectinase enzyme, if added in limited quantity, may be rendered inert by some action of living tissue. In a comparative study of *Bacillus carotovorus* and *Pythium de Baryanum* which attack potato tissue and of a strain of *Botrytis cinerea* which does not, results of the type summarized in Table I are obtained. The tissue of potato tubers has normally a certain avidity for water; such tissue is referred to in the Table as "subturgid." Previous to the experiment the enzymic preparations are tested by a standard method and their activities adjusted to the same value. Equal small quantities are then placed upon cylinders of potato tissue.

TABLE I

	<i>Tissue subturgid</i>	<i>Tissue turgid</i>
<i>Botrytis</i> spores	No attack	Attack
<i>B. carotovorus</i> suspension	Attack	"
<i>P. de Baryanum</i> mycelium	"	"
<i>Botrytis</i> enzyme	No attack	"
<i>B. carot.</i> "	Attack	"
<i>Pythium</i> "	"	"

The same result is obtained even when the *Botrytis* enzyme is definitely more active, as tested by the standard method (on turgid tissue), than those of the other two organisms. The enzymes of *B. carotovorus* and *Pythium* are more active upon the turgid than on the subturgid tissue, as likewise the organisms, but the signifi-

cant point is that while a certain degree of lack of turgor slows down the activity of *B. carotovorus* and *Pythium* and of their enzymes, the same inhibits *Botrytis cinerea* and its enzyme. The striking parallelism between the behavior of organism and enzyme gives grounds for the belief that the mode of action of the organism will be much illuminated when the mode of action of the enzyme is better understood. The enzyme theory manifestly involves many complications, but one could hardly expect that a complicated set of phenomena would be explainable on any simple theory.

Leaving now the subject of chemical means of attack, one cannot ignore the possibility that fungi may be able to progress through a tissue by mechanical action alone. Cell walls might be pierced by mechanical means and the mere presence of a foreign body, such as a hypha, within the protoplast might conceivably lead to death of the latter. The extent to which mechanical or chemical factors come into play in the progress of invasion probably varies very much in different instances. Where there is pronounced rotting of the tissue, chemical action is no doubt predominant, and conversely, where microscopic study shows that the fungus has traversed cell walls by means of fine penetration hyphae, one is justified in speaking of mechanical penetration. There are some references in the literature to the correlation of resistance with mechanical hardness of tissue or with average thickness of cell walls—as in the case of plum varieties to *Sclerotinia cinerea* (144) and of potato varieties to *Pythium de Baryanum* (65). On the other hand, bacterial parasites, for which it is difficult to postulate a capacity to penetrate cell walls mechanically, do freely permeate the tissue of the invaded plant, thus indicating that the process of invasion within the tissue can be effected by chemical means alone. The same explanation would apply also to the penetration of cells of potato tissue by the plasmodium of *Spongospora subterranea* as described by Kunkel (84).

The aggressive mechanism of the parasite having now been discussed, it remains to consider the nature of the resistance offered by the plant. This falls under the usual two headings, mechanical and chemical.

Layers of cork formed some little distance in advance of invading hyphae are the most familiar examples of the mechanical type of resistance. These are constant features in certain diseases,

e.g., in apple-scab and in the corky-scab disease of potato, at least in the milder form of the latter. In both cases the cork barriers are considered to function in preventing the invasion of the deeper tissue layers by the parasite. In a study of the resistance of different varieties of pear to *Bacillus amylovorus*, Shaw (121) has found that the more resistant varieties are characterized by a more rapid formation of cork round the bacterial lesions and Fahmy (41) states that the recovery of cotton plants from attack by *Rhizoctonia solani*, which is brought about by raising the temperature, is accompanied by the walling off by a corky layer of the cavity formed by the fungus. Nevertheless, there has always been some doubt as to whether cork barriers really function at all or merely mark the limit of spread of the parasite which has already been stopped by some chemical factor. Thus, Cunningham (30) reports that the margins of lesions caused by certain leaf-spotting fungi may or may not show corky barriers, though the advance of the parasite is equally arrested in both cases, and Thomas (128) notes that cork barriers may actually be penetrated by the hyphae of *Armillaria mellea*. The same considerations apply to the gum barriers which are associated with the resistance of certain plum varieties to silver-leaf disease (13) and of lettuce to *Botrytis* disease (1).

The formation of cork or of gum is a wound reaction which is characteristic of certain plants and which may be modified by the conditions prevailing, e.g., by aeration, by humidity, by the vigor of growth of the plant, etc. It is not clear whether fungal secretions influence the formation of cork or gum; whether, for example, a rampant parasite suppresses the natural tendency of the plant to lay down such barriers, or conversely, whether a fungus which has been arrested in its progress has contributed to that end by intensifying the natural response of the host. That the excretions of some fungi intensify the formation of wound gum has been suggested in Willison's (145) study of certain peach diseases.

Cells with lignified or otherwise altered walls are, in general, resistant to invasion, and in some cases the presence of such cells limits the area of spread of the parasite. Perhaps the most familiar examples of this structural type of resistance are found among the obligate parasites, e.g., as seen in the line-like sori of some rusts (*Puc. glumarum*) or smuts (*Ustilago longissima*) on

grasses, where lateral spread of the fungus is prevented by the parallel strands of fibro-vascular bundles. Within the group of facultative parasites, the effect is well shown in the lesions produced on cotton leaves by *Bact. malvacearum* whence is derived the common name "Angular leaf-spot" disease of cotton. The endodermis, with its walls thickened on the radial surface, is likewise in some cases a layer resistant to invasion, as has been shown by Pearson (108) for varieties of corn resistant to *Gibberella saubinetii*.

The influence of chemical factors in determining internal resistance has been much investigated. An obvious line of research is to attempt to demonstrate, in the sap of resistant plants, the presence of substances inhibitory or toxic to fungi. Thus the occurrence of tannin (which is somewhat toxic to fungi) in the outer layers of plants has been considered (29) to predispose to resistance. Schmidt (120) finds that species of *Solanum* resistant to *Cladosporium fulvum* contain in their sap a principle antagonistic to the fungus. There was some indication that the active ingredient was the alkaloid solanin, but Schmidt found it necessary to postulate a hypothetical substance "prohibitin" of unknown composition. According to Reynolds (113), there is a higher percentage of a glucoside, which gives hydrocyanic acid on hydrolysis, present in flax strains which are resistant to *Fusarium lini*. Spores of *Fusicladium dendriticum* germinate better in the sap of susceptible than of resistant varieties of apple (146, 79) but curiously the amount of germination is better still in pure water. Thus one must conclude that susceptible varieties are attacked in spite of some antagonistic principle in their sap. This principle (79) is most abundant in trees deficient in nitrogen, which are known to be the most resistant, but it occurs in quantity also in young leaves and fruits, which are quite susceptible. The relation between resistance and composition of sap is, therefore, not very clear in this case.

Perhaps the most striking evidence of the association of resistance with the presence of a toxic compound is that furnished by Link and Walker (91) in their studies of the "smudge" disease of onions caused by *Colletotrichum circinans*. It is well known that raw onion juice contains a volatile principle which is distinctly toxic to fungi, but less so to those which parasitize onion. Over

and above that, these workers have shown that the sap of colored resistant varieties is more toxic to *Colletotrichum* than is that of white susceptible varieties. This greater activity has been proved to arise from the presence of two aromatic compounds, proto-catechuic acid and catechol.

Acidity of the cell sap is claimed to determine the resistance of some plants. Thus Horne (70) associates the diminishing resistance of apple fruits which takes place during the process of ripening with progressive diminution of acidity. Similarly, Dickson, Link and Dickson (35) find that the embryonic tissues of corn varieties which are resistant to *Gibberella saubinetii* have a high acidity due to the presence of sugar acids.

An interesting type of chemical resistance is that in which the factor responsible is the low concentration of a sap constituent. Horne (69), for instance, has shown that there is a high correlation between low soluble nitrogen content of apple fruits and resistance to a number of parasites and this has been confirmed by Vasudeva (136) who finds that the invasion of apple fruits by such parasites as *Botrytis cinerea* and *Monilia fructigena* is much intensified by the supply of a trace of soluble nitrogen in the inoculum; and, furthermore, that a similar addition renders *Botrytis allii*, a non-parasite of apple, capable of attack. Apple fruit is very deficient in soluble nitrogen and Vasudeva explains the results just described on the ground that low nitrogen depresses the secretion of pectinase enzyme by the fungi. Conversely, a high nitrogenous content predisposes to attack, as has been shown by Böning (10) for *Bacterium tabacum* on tobacco leaves and by Fehmi (43) for a number of rotting organisms on potato tubers.

An interesting elaboration of the tannin hypothesis of Cook and Taubenhaus (see above) has been propounded by Dufrénoy (37) in a series of papers. This author has examined cytologically the effects of a number of parasites (obligate as well as facultative) upon plant tissues and claims that resistance arises from the accumulation, in cells bordering the lesion, of tannins or other phenolic derivatives. The accumulation of an excessive amount of these toxic substances is stated to cause the death of the plant cells and also of the parasite. The evidence is based on staining reactions of vacuolar contents, and while the appearance described seems to be widespread and on that account must be allowed to be

of importance, it is not easy to discover any solid basis for the conclusions drawn. Certain dark staining masses are stated to be phenolic compounds and the concentration of these is said to be sufficient to kill host-cells and parasite. Clear evidence bearing on each of these points is required. In so far as Dufrénoy's work explains the arrest of fungal lesions, two criticisms of an *à priori* nature may be offered. In the first place, if the mechanism of arrest is as stated, it is difficult to understand why the lesion ever progresses at all beyond microscopical dimensions, and in the second place, it is open to question whether, at the time a fungal lesion has reached its utmost limits, the hyphae of the parasite are in fact dead. In the light of what is known about the growth of fungi in culture, it is more plausible to suggest that the arrest of a fungal lesion, if it is not due to an environmental change which is unfavorable to attack, is to be explained by the stalling effect of the products of fungal metabolism.

While examples of antagonistic chemical factors could no doubt be multiplied, many investigators have failed to detect any correlation between resistance and the acidity or any other property of the cell contents, and one may venture to forecast that this will prove to be the general rule. At the best, one can only visualize inhibitory or toxic factors as restricting the number of parasites possible. Thus it is understandable that only fungi (e.g., *Monilia fructigena*) which prefer or at least tolerate a high degree of acidity could parasitize very acid tissues (such as young apple fruit). At the same time there is a vast number of plants with sap acidity which is well within the range of a large number of parasitic fungi—which nevertheless may be unable to parasitize these plants; and the same applies to other toxic or inhibitory constituents of the cell sap. What mechanism, therefore, can be suggested to explain a type of chemical resistance which is independent of substances antagonistic to fungal growth? Two lines of research may be quoted in this connection.

The behavior of potato tissue to *Botrytis cinerea*, to which reference has already been made, illustrates a type of resistance which is probably of very wide occurrence. Here there is nothing in the composition of the tissue which is harmful to the fungus. The spores germinate strongly in extracts of the tissue by whatever method these are prepared. These extracts are entirely suitable

for the development by the fungus of its offensive mechanism. Nevertheless, there is no attack. As was pointed out above, the line dividing attack from non-attack is very narrow, since a slight increase in the water content changes the tissue from resistant to susceptible. The explanation is probably to be found in the mechanism of enzyme action, as was indicated above.

Though it is not proposed in this article to deal at length with the effect of environment on disease, a few words may appropriately be said at this point on the effect of water relationships. That a high degree of humidity is generally a factor which favors plant disease has been long known, but the usual idea is that humidity is important chiefly in the phase before penetration. The discussion of the foregoing paragraph indicates how reduced water content may contribute to resistance by its effect upon the enzymic mechanism. A number of statements in the literature clearly indicates the close relationship between high water content and susceptibility; e.g., of pear to *Bac. amylovorus* (121), of tobacco leaves to *Bact. angulatum* and *Bact. tabacum* (28) and of potato leaves to *Phytophthora infestans* (101). It is probably for the same reason that certain workers, e.g., Young (149), have found that under artificial conditions of inoculation a number of fungi attack hosts upon which they do not occur naturally, so that one may speak of "new diseases" produced in the laboratory.

The second line of research, which considers a type of chemical resistance which is independent of plant toxins, is that of Dickson and his collaborators (34, 36) on the reaction of wheat and corn varieties to *Gibberella saubinetii*. The same strain of this organism attacks varieties of both wheat and corn, but with the difference that wheat is attacked at high but not at low temperatures, whereas with corn the converse relationship is shown. Dickson's work indicates a striking correlation between resistance and a type of metabolism which is in the main determined by the prevailing temperature. At low soil temperatures, the starch of the wheat endosperm is hydrolyzed much more rapidly than is the protein, with the result that the seedling is rich in sugar but poor in nitrogen. The cell walls, therefore, thicken rapidly by the deposit of cellulosic material upon the original pectic framework, and on that account become much less susceptible to fungal attack. On the other hand, at high temperatures both the starch and the protein

are rapidly hydrolyzed, the seedling is definitely richer in soluble nitrogen, growth is much more rapid and the cell walls remain much longer in the primary pectic condition. Hence the greater susceptibility to fungal attack. The behavior of corn seedlings in relation to temperature is the converse of that described for wheat. At high temperatures (which are favorable to the corn plant) the cell walls of the seedling are of a resistant and somewhat suberized type, whereas the unmodified pectic type of cell wall is produced at low temperatures.

More recently Gäumann (54) has confirmed that the cell walls of wheat germinated at a high temperature are more easily hydrolyzed by *Fusarium herbarum* than those of plants grown at a low temperature, and that the curve of digestibility of the cell walls runs parallel to the curve of their pentosan content. These investigations all point to the importance of cell wall composition in determining resistance and reinforce the writer's view of the importance of the pectinase enzyme in parasitism.

The discussion of the facultative type of parasite has so far referred to the kind of disease in which the effects upon the host are more or less confined to the neighborhood of the parasite. There is, however, a well known class of diseases in which the main symptoms tend to be shown by the whole plant and at parts more or less remote from the location of the parasite. These are the important wilt diseases, which now call for some special consideration.

The essential feature of a wilt disease is that the parasite enters the root system and after traversing the cortex, in which it may produce relatively little disturbance, it reaches a striking development in the vascular system. The functioning of the latter is interfered with, and as a result the shoot wilts and the whole plant dies. All manner of views have been expressed as to the cause of wilting and killing. The earliest investigators (123) suggested that the cause was simply mechanical blocking of the water-conducting system by the fungal mycelium. Further study of the many naturally occurring wilts showed that this explanation was inadequate, for in many cases there is relatively little mycelial development within the vessels. While theories of mechanical blockage (by tyloses, gum formation) have not been altogether abandoned, the general tendency has been to search for a toxic sub-

stance which is excreted by the fungus into the water stream of the plant. The method of experiment has been somewhat as follows.

Cultures of the parasite are set up on a liquid medium* and after a certain time the fungal mat is filtered off and the filtrate examined for toxic substances by placing in it whole plants or cut shoots of the host. Parallel controls are set up with water or with various strengths of the original culture medium. Extracts of the mycelial mats have been similarly tested. The presence of a toxin is shown by the wilting of the test plants.

A selected list of the conclusions which have been reached by various workers is set out in Table II, from which it will be seen

TABLE II

<i>Host: Parasite</i>	<i>Cause of Wilting</i>	<i>Author</i>
Melon, <i>Fusarium nivium</i>	Tyloses	Sleeth (122)
Tomato, <i>Bact. solanacearum</i>	Gum formation	Van der Meer (133)
Cotton, <i>F. vasinfectum</i>	Rotting of roots	Fahmy (40)
" " "	Physiological;	Dastur (32)
" " "	Aluminum salts	
" " "	from soil	Rosen (117)
" " "	Volatile alkaline	
" " "	substance + nitrite	Neal (102)
" " "	Thermolabile	
" " "	substance	Fikry (44)
" " "	Thermostable	
" " "	substance which	Schaffnit and Lüdtke (118)
" " "	is not nitrite	
" " "	Amines	"
Tomato, <i>F. lycopersici</i>	"	White (141)
" " "	Enzyme + volatile	
" " "	substance + ther-	Luz (93)
" " "	mostable sub-	
" " "	stance	Van der Veen (134)
" <i>Verticillium albo-atrum</i>	? Ammonia	
Flax, <i>F. lini</i>	Nitrite	Tochinai (131)
" " "	CO ₂ gas bubbles	Grossmann (60)
" " "	Thermostable	
Banana, <i>F. cubense</i>	substance	Lathrop (85)
" " "	Aldehyde	
Sugar cane, <i>Helminthosporium</i>	Thermostable	Lee (88)
<i>sacchari</i>	substance;	
" " "	? nitrite	Brooks & Brenchley (12)
Plum, <i>Stereum purpureum</i>	Enzyme + thermo-	
" " "	stable substance	

* Richards' solution (cane sugar 50 g; KNO₃, 10 g.; KH₂PO₄, 5 g.; MgSO₄, 2.5 g.; FeCl₃, trace; water, 1 litre) or some modification of it (e.g., glucose in place of cane sugar) has been much used in these studies.

how very diversified are the views expressed. Included in the table are two diseases (the last two) which are not classed as wilts, but as they both show the same "action at a distance" and as the mechanism of this action has been studied along the lines indicated, their inclusion in the table is quite appropriate.

Some of the work has been carried out with great care, e.g., effects which might arise from changes of H-ion concentration in the culture medium have been eliminated, and the test solutions have been freed from fine suspensions (which might block the vessels) by filtration through porcelain. It is difficult, therefore, to avoid the conclusion that destructive substances are present in the fungal filtrates. Very few workers, however, have studied the effects on the plants in detail, and it is not clear whether the action is on the vascular elements or on the living cells of the leaf. It would be interesting in all cases to know whether the wilted shoots were in fact poisoned or were merely wilted on account of some interference with the ascent of water.

Some workers, e.g., Haymaker (66), claim that the action of the toxin is specific and closely parallels that of the fungus on the host. Thus the filtrates of virulent strains of the fungus are the most active, and susceptible hosts are more sensitive to the toxin than resistant ones. This view, however, is exceptional and the general consensus is that there is no specificity. Not merely do resistant varieties succumb to the toxin as readily as do susceptible varieties, but so also do plants which are in no way connected botanically with the particular host species. This lack of specificity is not in itself a real objection to the toxin theory as it is quite plausible that wilt resistance is based on some character of the host other than resistance to the toxin. Perhaps a more serious objection to the toxin theory is contained in the results of Barnum (5) who finds that the same wilting effects are produced by filtrates of *Penicillium expansum*, which is not a wilt-producing fungus.

The production of toxins in culture is not an invariable feature of a wilt-producing fungus, as was recognized by Rosen (*l. c.*), one of the earlier protagonists of the toxin theory. He found that the cotton-wilt fungus gave toxic filtrates when grown in Richards's solution but not in Uschinsky's solution or in a number of natural decoctions. In this connection he writes: "This

opens up the whole question as to the significance to be attached to findings of toxic properties with any medium which does not closely approach the chemical and physical make-up of the natural host." In the light of this statement it is curious that he bases his claims on filtrates obtained from Richards's solution, which is not obviously a close approximation to the sap of the root cells or of the vascular system. This question of toxins and the relevancy of the findings of cultural experiments has already been considered for a simpler type of host-parasite relationship. In the case of wilt diseases there is the further complication that it is not clear which living cells in particular are being poisoned by the toxins, whether cells of the root cortex, or living cells associated with the vascular system in the stem or the mesophyll cells of the leaf. It should be possible to provide evidence on these points, but there would still remain the serious difficulty of trying to understand the breakdown of a process, viz., the ascent of sap, when plant physiologists have not yet produced a completely satisfactory theory of its mechanism in the normal plant.

The toxin theory of wilt diseases loses in cogency from the multiplicity of toxic agents suggested (Table II) and from the claims that fungi which do not cause wilting diseases, nevertheless, produce toxins. It is not improbable that the mechanism envisaged, viz., the excretion by the fungus of a toxin or toxins into the vascular stream, presupposes an over-simplification of the problem. Wilt diseases are not at all uniform in kind. In some there is apparently little damage to the root cortex, but in others there is very definite root rotting, and there is in fact no hard-and-fast line between a wilt and a root-rot or foot-rot disease. The same organism may cause a cortical rotting of a young plant and a wilt of an older plant. The "action at a distance" feature is not always shown as some wilt-producing fungi are not confined to the lower parts of the plant. The wilting and killing of the host in some cases follow soon after inoculation, in others after a very long interval. Thus in the carnation wilt caused by *Verticillium cinerescens* (142) the incubation period may extend to many months and the whole plant may be permeated by the fungus before any external symptoms are shown. With such a variety of host-parasite relationships grouped together under the name of wilt diseases, it would not be at all surprising if the causes of wilt-

ing and death were somewhat different in different instances. Linford (90) has clearly shown the necessity of a much broader physiological treatment of the problem than would arise from the assumption that the action of the fungus is primarily on the water-conducting system.

One leaves the toxin theory of wilt diseases with the certainty, therefore, that no clear indication has been given of the nature of the toxin and with a suspicion that the complexity and heterogeneity of wilt diseases have not been fully appreciated.

Whatever the nature of the offensive mechanism of the parasite, it is generally agreed that the resistance of the host lies in the cortical cells of the root. This has been demonstrated, for example, by Tisdale (130) for flax wilt and by Jones (80) for the bacterial wilt of alfalfa.

THE OBLIGATE PARASITE

The contrast between a facultative parasite like *Botrytis cinerea* and an obligate parasite such as *Puccinia graminis* is very pronounced, and is as shown in the scheme below:

<i>Botrytis cinerea</i>	<i>Puccinia graminis</i>
Host range wide	Host range narrow.
Hyphae inter- and intracellular	Hyphae intercellular with intracellular haustoria.
Tissues of host killed in advance of fungal growth, i.e. no symbiosis shown	Host tissue not killed until a late phase, symbiosis shown at first.
Parasite readily cultivable on artificial media ..	Parasite non-cultivable on artificial media.

A further general feature of the obligate parasite, though this is not shown by *Puccinia graminis* on its grass host, is the tendency to gall-formation, which is not nearly so marked with fungi of the type of *Botrytis cinerea*.

None of the differences listed above, however, is hard-and-fast and many intermediate conditions are known. As regards host range, some rust fungi are much less specialized (e.g., *P. malvace-*

arum, which ranges over several genera of the Malvaceae), and the parasitic range of the *morphological species* may be very wide, as in *Erysiphe polygoni* which attacks *Polygonum*, clover, pea, swede, etc. It is true that the species in the latter case consists of a number of biological races, each with a comparatively circumscribed host-range but the same is to a large extent true of typical members of the facultative group. Even *Botrytis cinerea* and *Rhizoctonia solani*, which are stock examples of the "plurivorous" type of parasite, consist of a large number of distinct races (or even subspecies) each of which has a much narrower range than that of the "group species." Then again forms like *Ophiobolus graminis*, *Venturia inaequalis*, *Cladosporium fulvum* and many others, which are classed as facultative parasites* are as narrowly restricted in their parasitism as many members of the obligate group.

The relation of the mycelium of the parasite to the host cell gives one of the best criteria for distinguishing the two types. Where the thallus of the fungus is entirely intracellular (as in *Synchytrium* or *Plasmodiophora*) or where haustoria are the only intracellular structures (as in some downy mildews and smut fungi, in all powdery mildews and rust fungi) one may be certain that the parasite is of the obligate type.

Early and rapid killing of the host tissue is not always so pronounced as with *Botrytis cinerea* and in some cases killing is slow and not at all distinct at first. There are thus intermediate types which lead up to the condition shown by *Puccinia graminis* when growing in symbiosis with its appropriate host. Conversely, the "hypersensitiveness" shown by a host plant to certain strains of a parasite has been regarded as an effect peculiar to obligate parasitism, but it is claimed that something very similar occurs with *Colletotrichum lindemuthianum* or certain varieties of *Phaseolus* (86).

Non-cultivability of the parasite is associated with the haustorial habit. No great emphasis need be attached to this negative character, as it merely means that no one as yet has discovered the secret of a highly specialized type of metabolism. At the moment,

* These are more appropriately described as "facultative saprophytes," in the sense that they have regularly parasitic phases but are able to live saprophytically in the intervals.

however, one can safely say that no fungus which possesses clearly defined haustoria has been cultivated on an artificial medium, that is, to any extent beyond mere spore germination. But here again there are intermediate states. Between the obligate parasite and the kind of fungus which is cultivable on any medium within reason, many intermediates may be noted. Thus *Exoascus*, which attacks its hosts after the manner of an obligate parasite, can be maintained in culture but almost wholly in a yeast-like form which is very different from its parasitic mycelium. Then among facultative parasites there are wide differences in the ease with which they can be cultivated. Some require special media, and if one is aiming at a complete life cycle in culture, with the characteristic fruiting structures such as perithecia, etc., the number of difficult subjects is very considerable.

Finally, the feature of gall formation is not confined to the obligate parasites and is not invariable in their case. Thus a distinct gall is formed round the lesion caused by *Nectria galligena*. *Fusarium moniliforme*, a fungus with pronounced killing tendencies, causes a peculiar "overgrowth" of the invaded plants as a preliminary symptom; and most striking of all, the crown-gall organism which is a readily cultivated parasite of wide host-range produces on its hosts large and characteristic galls.

The fact that the obligate parasite cannot be cultivated apart from its living host forces upon the investigator an indirect method of studying its physiological relationships. Its metabolism being quite unknown, one has to fall back upon microscopic examination the results of which may in part be interpretable in terms of physiology, and upon a study of the host's metabolism in its relation to the progress of the disease. The cytological relations of host and parasite have already been dealt with in this REVIEW (114). It now remains to consider the problem from the second point of view.

Most of the work in this connection has been carried out with rust fungi and more particularly with forms of *Puccinia* on cereal hosts. The method adopted is to study the effect of various environmental factors upon the grade of infection. Maximum susceptibility is correlated with best development, both as regards number and size of the spore masses, and with a minimum period from inoculation to sporulation. When the host is resistant, sporulation,

if it occurs at all, is scanty and the spore masses are small; there is also in many cases a development of minute dead spots over the surface of the host, this being the outward expression of "hypersensitiveness." A series of infection types, ranging from highly susceptible to highly resistant or immune, has been distinguished by various workers on the basis indicated.

The general effect of *light* upon the host is to increase susceptibility, though the existence of an optimum period of illumination has been indicated (8). Forward (45) states that for *Puccinia graminis tritici* the uredo-pustules are fewer in number and slower in development when the host is kept in darkness, and that in poor light there is a tendency towards hypersensitiveness in hosts that are normally quite congenial to the parasite. Similar results have been obtained by Gassner (48), Johnson (75) and Wilhelm (143) for various cereal rusts and by Waters (140) for bean rust. The association of susceptibility with good conditions for assimilation is further supported by Hanna's (61) statement that wheat varieties which are highly susceptible to *Puccinia graminis* are characterized by a relatively high content of the green and yellow chlorophyll pigments.

In this connection it is interesting to note the behavior of the nodule bacteria of lucerne and clover when the host plant is kept in darkness. Under such conditions the bacteria do not set up the normal symbiotic relationship but actively parasitize the root cells (129).

The influence of *temperature* upon the infection type has been studied for a number of rust fungi, but with no clear-cut result. In many cases, change of temperature produced no effect, and where there was a change in host reaction it was not always in the same direction for the different diseases studied.

Considerable attention has been paid to *mineral nutrition* as a factor influencing susceptibility, and the general result has been to confirm the earlier work of Spinks (125) on this subject. High nitrogenous manuring, according to Spinks, increases the susceptibility of wheat and barley to their particular rusts and mildews, whereas potash acts in a contrary manner, though high potash does not completely counteract the effect of high nitrogen. Conversely, plants with very low nitrogen are resistant even with minimal potash. The subject has been recently investigated in

great detail by Gassner and coworkers (50, 51, 64), by Schaffnit and Volk (119), and by some others, with results which are in essential agreement with the above. The effect of phosphorus is not always clearly marked; in some cases it acts in the same manner as potash and in others not.

An interesting observation made by Spinks but which does not seem to have attracted much attention, is that the addition of lithium salts markedly increases rust resistance.

The effect of *carbon feeding* runs parallel, as might be expected, to that of light. By placing the host plants (oats and wheat) in an atmosphere devoid of carbon dioxide, Gassner found that the development of the corresponding rust uredospores was inhibited. Gassner and Straib (49) found that the optimum concentration of carbon dioxide for the development of a number of rusts ranged from .15% to .75%. Concentrations of that order had no effect on the germination or growth of uredospores, so that presumably the effect on the disease arose from the action of the carbon dioxide upon the host. Results which point in the same direction have been obtained in another way. Waters (140) tested the susceptibility to *Uromyces fabae* of bean leaves which were fed with various sugar solutions, and found that uredospores were formed freely when the leaves were floated in the dark on a 5% sucrose solution whereas none were formed when the leaves were floated on water. This result has been confirmed by Yarwood (148), working with the rust and mildew of clover. The latter also states that detached leaves are more susceptible if taken from the plant in the late afternoon, when they are fully charged with carbohydrates than when taken in the early morning. All these statements support the view that active carbon assimilation increases susceptibility to the obligate type of parasite.

The age of the host plant has an effect, within limits, upon disease response and there is general agreement that resistance increases as the plant grows older. Hence arises the "mature plant resistance," the effect of which is to make a particular variety of the host resistant to strains of *Puccinia graminis tritici* to which it is susceptible in the seedling stage (55, 56). Similar results have been obtained for the crown and stem rusts of oats (38) and for brown rust of wheat (78). According to Gassner (52), *Puccinia triticina* conforms with the rule as stated, whereas *P. graminis avenae* and *P. lolii* behave in the converse manner.

A striking statement in this connection is made by Newton and Brown (193) who injected uredospores of *P. graminis* into the young and still enclosed leaves of various cereals. Under these conditions not merely are the very young leaves of resistant varieties highly susceptible, but the young leaves even of oats and rye are attacked by forms of *P. graminis tritici*.

The composition of the host has been studied from many points of view, with results that are in many cases negative or indefinite. Acidity of the cell-sap does not seem to be related in any way to susceptibility (72, 126, 73). Newton, Lehmann and Clarke (104) find no correlation between resistance and various properties of the cell sap such as osmotic pressure, electrical conductivity, H-ion concentration, total solid content or amount of bound water. Even the sugar content does not appear to vary consistently with variations of resistance. Thus Schaffnit and Volk found that the susceptible type of plant which resulted from over-manuring with nitrogen had a high sugar content, and conversely for the resistant nitrogen-starved plant. Nevertheless, low potash gave a type of plant which had a low sugar content but which was highly susceptible. As from one variety of wheat to another, Johnson and Johnson (76) have found no relation between resistance and the sugar content of the sap. Ezekiel's (39) claim that better germination of uredospores takes place in the sap of susceptible than of resistant varieties has not been borne out by the later work of Anderson (2). From a study of the reaction of wheat to a number of rusts at different temperatures, Gassner and Franke (53) find that increased susceptibility runs parallel with the concentration of protein nitrogen, thus supporting the earlier statement of Honecker (68) that resistance of barley to *Erysiphe graminis* is correlated with low albumin and abundant starch.

The presence of toxins in resistant varieties has been advocated by a number of workers. Thus Kharbush (82) considers that resistance of wheat to *Puccinia glumarum* is due to histolysis of the chloroplasts, leading to the absorption of a toxic substance by the fungus. This worker supports Dufrénoy in stating that resistance is associated with the accumulation of phenolic compounds in the vacuoles of cells bordering the invaded region, and more recently Newton and Anderson (105) have expressed the same view. Similarly, Rochlin (116) claims that there is a direct connection

between the resistance of various crucifers to club-root disease and the amount of glucosides in the roots which on fermentation give rise to pungent mustard oils.

Though the effect is entirely beyond explanation, it is interesting to note that the resistance of a plant to a specialized parasite may in some cases be broken down by the presence of another parasite. Thus the presence of the endo-parasite *Tilletia tritici* increases the susceptibility of wheat to *Puccinia glumarum* (137). Similarly, the resistance of Warden wheat to *P. tritici* is lost in the presence of *Erysiphe graminis* (77). Among facultative parasites the same effect is known, as in the diminished resistance to blight of potato plants which are infected with virus disease.

Whatever be the chemical substances which determine host resistance or susceptibility, whether toxic substances which act against or food substances which act in favor of the parasite, it is quite clear from a number of researches that those substances do not freely move about the plant. This is true not only for obligate but also for facultative parasitism. Thus Leach (87) has shown that when a variety of *Phaseolus vulgaris* susceptible to *Colletotrichum lindemuthianum* is grafted on a resistant one, the two components react in the normal manner, thus showing that organic continuity has not led to the interchange of substances which affect resistance. The same result has been obtained by May (95) and by Roach (115) for the resistance of tomato to wilt and of potato to wart disease, respectively. But perhaps the most striking evidence in this connection is obtained from infection experiments with graft hybrids (83), where it is found that the layers of the composite plant react to specific parasites exactly as does the tissue of the species from which they originated. Even such an intimate association of tissues as occurs in graft-hybrids does not lead to any interchange of substances which materially affect disease response.

So far for the effect of the metabolism of the host upon the reaction of the obligate parasite. When we come to consider the mechanism of the parasite there is little to say and that little is derived from cytological observation. Penetration of cell walls is apparently by mechanical means only as there is no trace of a chemical action upon the walls and the neck of the haustorium is always a slender thread-like structure, such as one associates with mechanical penetration. It should be noted that the effect of potash in increasing

disease resistance may be partly due to its action in intensifying cell wall development. Lowig (92), for example, has found potassium silicate to be the most effective salt in increasing the resistance of cereals to mildew attack and correlates this behavior with the mechanical resistance of the epidermal wall. Of enzymes or toxins which act upon the protoplasts there is no direct evidence but one may surmise that hypersensitiveness has its basis in a toxin either excreted by the fungus or liberated from the host through an action of the fungus.

CONCLUSION

This review of the physiology of the obligate parasite-host relationship, which has unfortunately resolved itself largely into a catalogue of negatives, may be concluded best perhaps by raising a number of general considerations which, though they may not throw any light upon the problem at the moment, may indicate where a solution is to be sought with greater prospect of success. The cereal rusts have strongly attracted many workers, partly no doubt because of their great economic importance and partly because of the great variety of material (races of host and parasite) available for experiment. Furthermore, it is generally assumed that the relationship of the obligate parasite to its host brings in factors which do not exist in the simpler type. To illustrate, it is usual to compare the behavior of such a fungus as *Botrytis* on a susceptible plant with that of *Puccinia* on a resistant plant, and to state the position in this form: that a physiological relationship which leads to susceptibility with the one type of parasite leads to resistance in the other. Susceptibility to an obligate parasite would, therefore, be something which finds no parallel in the story of the facultative parasite, and so the tendency has been to emphasize the contrast between the two types. In particular it has been customary to ascribe the parasitic specialization of the obligate parasite to its nutritional specialization, e.g., to fine preferences for isomeric forms of carbohydrates or proteins. Such specialization as the facultative type of parasite shows must obviously rest upon grosser differences.

Now it is hardly possible to maintain a difference in kind between the two types of parasitic relationship, in view of the intermediate types which are known to exist. Even the symbiotic phase, which is the most striking feature of obligate parasitism, finds clear parallels

among the facultative group, as in the case of mycorrhiza, *Bacterium radicum*, etc. Again, the contrast between the obligate parasite which attacks its host most vigorously when the latter is growing under optimal conditions, and the facultative parasite which is favored by a weak condition of its host is definitely overdrawn. Certain forms of powdery mildew, for example, are favored by conditions (e.g., shading or droughting) which are far from optimal for growth of the host. It is even stated that local injuries predispose certain plants to mildew attack. Conversely, the statement that a weakened condition of the host predisposes to attack by facultative parasites is based on observations with certain types of the latter and it is certainly not true for many members of the group. Lastly, apart from a certain difference in degree, the phenomena of host specialization are the same in the two groups. One may, therefore, suggest that the key to the specialization of the obligate parasite may be sought for hopefully in a study of the facultative group. The latter have the enormous advantage of being cultivable, so that something may be learned about their metabolism. In particular, it may be possible to explain why some members of the group rapidly kill the invaded host cells whereas others do not. Information on that subject would go far to explain the symbiotic phase of obligate parasitism. It would even appear that gall formation could best be studied with the simpler type of organism, with *Bacterium tumefaciens* in particular. The physiological investigations with the *Puccinia* type of parasite, as reviewed above, have cleared up certain points and at least have served to define the problem, but they must necessarily be cramped by the non-cultivable nature of the parasite, and as such they do not, in the writer's opinion, represent a natural and scientific approach to the problem of selective parasitism.

Though it has been contended above that the specialization of the obligate parasite is not necessarily based upon its specialized nutrition, the latter is, nevertheless, an extremely interesting problem in itself, though unfortunately there is as yet no clue to its solution. Various chemical substances and plant extracts do in a somewhat irregular manner affect the vigor of spore germination, but no kind of medium has been found which is of value in giving sustained mycelial development. In speculating as to what might be lacking from the ordinary media, one would hardly suggest min-

eral elements as being of consequence, partly because they are present in the ionic form however supplied, and partly because fungi are able to sustain a reasonable amount of growth on the merest trace of mineral elements. The carbon food is also not likely to be the determining factor, as it is difficult to believe that glucose is not a suitable form of carbohydrate for all fungi. There remains the nitrogen constituent and there is in this case some evidence of specific preferences. Some fungi are able to assimilate nitrate, others not. Some prefer ammonium salts to nitrate, while others do not grow well except with organic form of nitrogen. The so-called "peptone" organisms apparently require complex forms of nitrogen, but as was shown by Farries and Bell (42) for *Nematospora gossypii* this selectivity is based, not upon the necessity for a protein as such, but upon growth-promoting substances which are associated with impure proteins. These growth-promoting substances are widely distributed in living tissues, are stable, diffusible and in the main non-specific. They may, therefore, be ruled out of account in the present connection as they occur in all natural decoctions and would be able to diffuse into the fungal hyphae. It appears, however, from recent plant physiological work, that a certain type of growth-promoting substance is readily deactivated by oxidation so that it does not occur in plant extracts unless certain precautions are taken (127). The functioning of such a labile substance offers a possibility which should not be ignored. Putting aside that possibility, one reaches the position that the obligate parasite requires to be supplied with nitrogen in its fully elaborated proteid form, and this approaches the view which is put forward by Gassner on the basis of his studies on rusts. On this view the haustorium would appear to be a structure which is permeable to a nitrogenous substance of high molecular weight, whereas the ordinary mycelium is not.

The speculations of the foregoing paragraph are based on the assumption that the obligate parasite is in fact highly specialized in its nutritional requirements, but it might be well to look at the evidence on which the current view rests. Briefly, it is that the germ tubes of obligate parasites do not respond to any known nutrient solution, whereas the parasitizing mycelium obviously obtains suitable food from the host. It is concluded, therefore, that certain food substances are made available by the living cells but that these are

not present in any extracts preparable from the latter. There is, however, another interpretation of the difference, which is that possibly the plant extract is quite suitable for growth *in vitro* but the fungus is presented in the wrong form—viz., devoid of haustoria. It is not unreasonable to suggest that if one could induce the formation of haustoria under artificial conditions, much of the so-called nutritional specificity might disappear. To be more explicit, one might suggest that the ordinary mycelium of the obligate parasite, being external to the plant cells, functions under strictly aerobic conditions and that the haustorium is a structure which is developed under reduced conditions of aeration (and perhaps in relation to some surface tension effect) and which absorbs food only under conditions of low oxygen tension. The latter suggestion is not so fanciful when one remembers that some enzymes are known which function only under these conditions. In this connection it might be interesting to study more fully the physiology of those fungi, e.g., certain Phycomycetes, which show a clear differentiation between an aerial and a matrical type of hypha.

A review of host-parasite relationships is not complete without some reference to the subject of induced or acquired immunity in plants. The orthodox plant pathologist is sceptical of the existence of such an effect and points out that, the plant body being organized on a basis essentially different from that of an animal, one cannot expect to find any close parallelism between plant and animal responses, and in particular that the phenomenon of generalized induced immunity cannot have a place in plant pathology. This view is supported by a vast amount of observational data showing that invasion of one part of a plant by a fungus in no way increases the resistance of other parts to subsequent reinfection. In recent years, however, some workers on virus diseases have described effects which at least closely simulate the effects which are well established by animal pathologists, and from time to time similar claims are made for plants diseases of fungal causation. Most of the latter claims relate to a localized acquired immunity, the inducing agent being some secretion of the fungus which is presumed to stimulate the host cells to a higher grade of resistance. The relevant literature has recently been reviewed in a favorable light by Chester (24). The subject is one of considerable complexity, for not merely are many of the results capable of more than one interpretation but there is still much

disagreement as to the results themselves. Any critical review of this subject would, therefore, of necessity be lengthy and would perhaps serve no useful purpose in the absence of general agreement as to what are fully substantiated data. The reader who is interested in this line of study will, therefore, be referred for further information to Chester's paper.

LITERATURE CITED

1. ABDEL-SALEM, M. M. Botrytis disease of lettuce. Jour. Pomol. & Hort. Sci. 12: 15-35. 1934.
2. ANDERSON, J. A. Studies on the nature of rust resistance in wheat. IV. Effect of hydrogen ion concentration, phenolic compounds, and host extracts on the germination of urediniospores of *Puccinia graminis tritici* form 21. Canad. Jour. Res. 11: 667-686. 1934.
3. ARENS, K. Physiologische Untersuchungen an *Plasmopara viticola*, unter besonderer Berücksichtigung der Infektionsbedingungen. Jahrb. Wiss. Bot. 70: 93-157. 1929.
4. BALLS, W. L. Infection of plants by rust fungi. New Phyt. 4: 18-19. 1905.
5. BARNUM, C. C. The production of substances toxic to plants by *Penicillium expansum* Link. Phytopath. 14: 238-243. 1924.
6. BARY, A. DE. Ueber einige Sclerotinien und Sclerotienkrankheiten. Bot. Zeit. 44: 377-474. 1886.
7. BEHRENS, J. Beiträge zur Kenntnis der Obstfäulnis. Centr. Bakt. Abt. II. 4: 547-553. 1898.
8. BEVER, W. M. Effect of light on the development of the uredial stage of *Puccinia glumarum*. Phytopath. 24: 507-516. 1934.
9. BLACKMAN, V. H. AND WELSFORD, E. J. Studies in the physiology of parasitism. II. Infection by *Botrytis cinerea*. Ann. Bot. 30: 389-398. 1916.
10. BÖNING, K. Beiträge zur Kenntnis des parasitischen Verhaltens von *Pseudomonas tabaci* Wolf et Foster, des "Wildfeuer" Erregers am Tabak. Zeits. Parasitenkunde. 2: 645-755. 1930.
11. BOYLE, C. Studies in the physiology of parasitism. VI. Infection by *Sclerotinia libertiana*. Ann. Bot. 35: 337-347. 1921.
12. BROOKS, F. T. AND BRENCHEY, G. H. Injection experiments on plum trees in relation to *Stereum purpureum* and silver-leaf disease. New Phyt. 28: 218-224. 1929.
13. ———. Silver-leaf disease. VI. Jour. Pomol. & Hort. Sci. 9: 1-29. 1931.
14. BROWN, W. Studies in the physiology of parasitism. I. The action of *Botrytis cinerea*. Ann. Bot. 29: 313-48. 1915.
15. ———. Ditto. III. On the relation between the "infection drop" and the underlying host tissue. Ann. Bot. 30: 399-406. 1916.
16. ———. Ditto. IV. On the distribution of cytase in culture of *Botrytis cinerea*. Ann. Bot. 31: 489-498. 1917.
17. ———. Ditto. VIII. On the exosmosis of nutrient substances from the host tissue into the infection drop. Ann. Bot. 36: 101-119. 1922.
18. ———. Ditto. IX. The effect on the germination of fungal spores of volatile substances arising from plant tissues. Ann. Bot. 36: 285-300. 1922.
19. ——— AND HARVEY, C. C. Ditto. X. On the entrance of parasitic fungi into the host plant. Ann. Bot. 41: 643-662. 1927.

20. BROWN, W. On the physiology of parasitism. *New Phyt.* 16: 109-127. 1917.
21. ———. Mechanism of disease resistance in plants. *Trans. Brit. Myc. Soc.* 19: 11-33. 1934.
22. CALDWELL, R. M. AND STONE, G. M. Appressorium formation and penetration by leaf rust of wheat, *Puccinia triticina*, in relation to stomatal aperture. *Phytopath.* 22: 5-6. 1932.
23. CHAUDHURI, H. Infection by *Colletotrichum gloeosporioides* Penz. *Proc. Nat. Inst. Sci. India.* 1: 71-75. 1935.
24. CHESTER, K. S. The problem of acquired physiological immunity in plants. *Quart. Rev. Biol.* 8: 129-154, 275-324. 1933.
25. CHONA, B. L. Studies in the physiology of parasitism. XIII. An analysis of factors underlying specialisation of parasitism, with special reference to certain fungi parasitic on apple and potato. *Ann. Bot.* 46: 1033-1050. 1932.
26. CLARK, J. F. Toxic properties of some copper compounds with special reference to Bordeaux mixture. *Bot. Gaz.* 33: 26-48. 1902.
27. CLAYTON, E. E. Toxin produced by *Bacterium tabacum* and its relation to host range. *Jour. Agr. Res.* 48: 411-426. 1934.
28. ———. A new and important factor in the epidemiology of tobacco leaf diseases. *Phytopath.* 25: 11. 1935.
29. COOK, M. T. AND TAUBENHAUS, J. J. The relation of parasitic fungi to the contents of the cells of the host plants. *Del. Agr. Exp. Sta. Bull.* 91. 1911.
30. CUNNINGHAM, H. S. A study of the histologic changes induced in leaves by certain leaf-spotting fungi. *Phytopath.* 18: 717-751. 1928.
31. CURTIS, K. M. The morphological aspect of resistance to brown-rot in stone fruit. *Ann. Bot.* 42: 39-68. 1928.
32. DASTUR, J. F. A preliminary account of the investigation of cotton wilt in Central Provinces and Berar. *Agr. Jour. India.* 19: 251-260. 1924.
33. DEY, P. K. Studies in the physiology of parasitism. V. Infection by *Colletotrichum lindemuthianum*. *Ann. Bot.* 33: 305-312. 1919.
34. DICKSON, J. G., ECKERSON, S. H. AND LINK, K. P. The nature of resistance to seedling blight of cereals. *Proc. Nat. Acad. Sci.* 9: 434-439. 1923.
35. DICKSON, J. G., LINK, K. P. AND DICKSON, A. D. Nature of resistance of corn to seedling blight. *Phytopath.* 23: 9. 1933.
36. DICKSON, J. G. AND HOLBERT, J. R. The relation of temperature to the development of disease in plants. *Amer. Nat.* 62: 311-333. 1928.
37. DUFRÉNOY, J. Observations sur les modifications pathologiques de la forme des vacuoles des cellules végétales. *Ann. Epiphyties.* 14: 227-268. 1928.
38. DURRELL, L. W. AND PARKER, J. H. Comparative resistance of varieties of oats to crown and stem rusts. *Iowa Exp. Sta. Res. Bull.* 62: 27-56. 1920.
39. EZEKIEL, W. N. Studies on the nature of physiologic resistance to *Puccinia graminis tritici*. *Minn. Agr. Exp. Sta. Tech. Bull.* 67: 62 pp. 1930.
40. FAHMY, T. Étude de la pénétration du champignon *Fusarium vasinfectum* (Atk.) var. *aegyptiacum* T. Fahmy dans les racines du cotonnier. *Geneva Univ. Thesis* 881: 70 pp. 1930.
41. ———. The sore-shin disease and its control. *Egypt Min. Agr. Plant Prot. Sect. Bull.* 108: 24 pp. 1931.
42. FARRIES, E. H. M. AND BELL, A. F. On the metabolism of *Nematospora gossypii* and related fungi, with special reference to the source of nitrogen. *Ann. Bot.* 44: 423-455. 1930.
43. FEHMI, S. Untersuchungen über den Einfluss der Ernährung auf die

- Empfänglichkeit der Kartoffelknolle für Lagerparasiten. *Phytopath. Zeits.* 6: 543-588. 1933.
44. FIKRY, A. Investigation on the wilt disease of Egyptian cotton caused by various species of *Fusarium*. *Egypt Min. Agr. Bull.* 119: 106 pp. 1932.
 45. FORWARD, D. F. The influence of altered host metabolism upon modification of the infection type with *Puccinia graminis tritici* p.f. 21. *Phytopath.* 22: 493-555. 1932.
 46. FULTON, H. R. Chemotropism of fungi. *Bot. Gaz.* 41: 81-108. 1906.
 47. GARRETT, S. D. Factors affecting the pathogenicity of cereal foot-rot fungi. *Biol. Rev.* 9: 351-361. 1934.
 48. GASSNER, G. Die Frage der Rostanfälligkeit als ernährungsphysiologisches Problem. *Angew. Bot.* 9: 531-541. 1927.
 49. ——— AND STRAIB, W. Untersuchungen über die Abhängigkeit des Infektionsverhaltens der Getreiderostpilze vom Kohlensäuregehalt der Luft. *Phytopath. Zeits.* 1: 1-30. 1929.
 50. ——— AND HASSEBRAUK, K. Untersuchungen über die Beziehungen zwischen Mineralsalzernährung und Verhalten der Getreidepflanzen gegen Rost. *Phytopath. Zeits.* 3: 535-617. 1931.
 51. ———. Ueber die Beeinflussung der Rostanfälligkeit durch Eintauchen geimpfter Blätter in Lösungen von Mineralsalzen und anderen Stoffen. *Phytopath. Zeits.* 5: 323-342. 1933.
 52. ———. Ueber Verschiebung der Rostresistenz während der Entwicklung der Getreidepflanzen. *Phytopath. Zeits.* 4: 549-596. 1932.
 53. ——— AND FRANKE, W. Ueber den Einfluss der Temperatur auf Stickstoffgehalt und Rostresistenz junger Getreidepflanzen. *Phytopath. Zeits.* 7: 315-326. 1934.
 54. GÄUMANN, E. Der Einfluss der Keimungstemperatur auf die chemische Zusammensetzung der Getreidekeimlinge. *Zeits. Bot.* 25: 385-461. 1932.
 55. GOULDEN, C. H. Breeding rust-resistant varieties of wheat. *Fundamental aspects of the problem.* *Scient. Agr.* 10: 258-267. 1929.
 56. ———, NEWTON, M. AND BROWN, A. M. The reaction of wheat varieties of two stages of maturity to sixteen physiologic forms of *Puccinia graminis tritici*. *Scient. Agr.* 11: 9-25. 1930.
 57. GRAF-MARIN, A. Studies on powdery mildew of cereals. *Cornell Agr. Exp. Sta. Mem.* 157: 48 pp. 1934.
 58. GRAVES, A. H. Chemotropism in *Rhizopus nigricans*. *Bot. Gaz.* 62: 337-369. 1916.
 59. GREEN, F. M. The infection of oranges by *Penicillium*. *Jour. Pomol. & Hort. Sci.* 10: 184-215. 1932.
 60. GROSSMANN, H. Untersuchungen über die Welkenkrankheit des Flachs. *Phytopath. Zeits.* 7: 545-583. 1934.
 61. HANNA, W. F. Studies on the nature of rust resistance in wheat. V. Physiology of the host. *Canad. Jour. Res.* 4: 134-147. 1931.
 62. HART, H. Relation of stomatal behaviour to stem-rust resistance in wheat. *Jour. Agr. Res.* 39: 929-948. 1929.
 63. HARTER, L. L. AND WEIMER, J. L. A comparison of the pectinase produced by different species of *Rhizopus*. *Jour. Agr. Res.* 22: 371-377. 1921.
 64. HASSEBRAUK, K. Ueber die Abhängigkeit der Rostinfektion von der Mineralsalzernährung der Getreidepflanze. *Angew. Bot.* 12: 23-35. 1930.
 65. HAWKINS, L. A. AND HARVEY, L. B. Physiological study of the parasitism of *Pythium debaryanum* on potato tuber. *Jour. Agr. Res.* 18: 275-297. 1919.
 66. HAYMAKER, H. H. Relation of toxic excretory products from two

- strains of *Fusarium lycopersici* to tomato wilt. Jour. Agr. Res. 36: 697-719. 1928.
67. HIGGINS, B. B. Physiology and parasitism of *Sclerotium rolfsii*. Phytopath. 17: 417-448. 1927.
 68. HONECKER, L. Beiträge zum Mehlaupproblem bei der Gerste mit besonderer Berücksichtigung der züchterischen Seite. Pflanzenbau. 8: 78-84, 89-106. 1931.
 69. HORNE, A. S. Biological work on fruit. Dep. Sci. & Indus. Res. Rep. Food Invest. Board. 125-140. 1930.
 70. ———. Ditto. Ibid. 272-289. 1932.
 71. HULL, K. L. Studies in resistance and susceptibility of *Zea mays* L. to *Puccinia sorghi* Schw. physiologic form I. (Abs.) Thesis Univ. Chicago Sci. Ser. 7: 317-320. 1931.
 72. HURD, A. M. The course of acidity changes during the growth period of wheat with special reference to stem-rust resistance. Jour. Agr. Res. 27: 725-735. 1924.
 73. JANCKE, O. Die Anfälligkeit verschiedener Pflanzen gegenüber tierischen und pflanzlichen Schädlingen und die Wasserstoffionenkonzentration ihres Zellsaftes. Phytopath. Zeits. 2: 181-198. 1930.
 74. JOHANN, H., HOLBERT, J. R. AND DICKSON, J. G. Further studies on *Penicillium* injury to corn. Jour. Agr. Res. 43: 757-790. 1931.
 75. JOHNSON, T. Studies in cereal diseases. VI. A study of the effect of environmental factors on the variability of physiologic forms of *Puccinia graminis tritici* Erikss. and Henn. Canad. Dept. Agr. Bull. 140 N.S.: 76 pp. 1931.
 76. ——— AND JOHNSON, O. Studies on the nature of disease resistance in cereals. II. The relationship between sugar content and reaction to stem-rust of mature and immature tissues of the wheat plant. Canad. Jour. Res. 11: 582-588. 1934.
 77. JOHNSTON, C. O. The effect of mildew infection on the response of wheat-leaf tissues normally resistant to leaf rust. Phytopath. 24: 1045-1046. 1934.
 78. ——— AND MELCHERS, L. E. Greenhouse studies on the relation of age of wheat plants to infection by *Puccinia triticea*. Jour. Agr. Res. 38: 147-157. 1929.
 79. JOHNSTONE, K. H. Observations on the varietal resistance of the apple to scab (*Venturia inaequalis* Aderh.) with special reference to its physiological aspects. Jour. Pomol. & Hort. Sci. 9: 195-227. 1931.
 80. JONES, F. R. Testing alfalfa for resistance to bacterial wilt. Jour. Agr. Res. 48: 1085-1098. 1934.
 81. JONES, L. R. The cytolytic enzyme produced by *Bacillus carotovorus* and certain other soft-rot bacteria. Centr. Bakt. Abt. II. 14: 257-272. 1905.
 82. KHARBUSH, S. Recherches cytologiques sur les blés parasités par *Puccinia glumarum*. Rev. Path. Vég. et Ent. Agr. 13: 92-110. 1926.
 83. KLEBAHN, H. Impfversuche mit Propfbastarden. Flora, Stahl's Festschrift. 418-430. 1918.
 84. KUNKEL, L. O. A contribution to the life-history of *Spongospora subterranea*. Jour. Agr. Res. 4: 265-278. 1915.
 85. LATHROP, E. C. The generation of aldehydes by *Fusarium cubense*. Phytopath. 7: 14-16. 1917.
 86. LEACH, J. G. The parasitism of *Colletotrichum lindemuthianum*. Univ. Minn. Agr. Exp. Sta. Tech. Bull. 14. 39 pp. 1922.
 87. ———. The effect of grafting on resistance and susceptibility of beans to *Colletotrichum lindemuthianum*. Phytopath. 19: 875-877. 1929.
 88. LEE, A. The toxic substance produced by the eye-spot fungus of sugar

- cane, *Helminthosporium sacchari* Butler. Plant Physiol. 4: 193-212. 1929.
89. LEPIK, E. Anatomische Untersuchungen über die durch *Plasmopara viticola* erzeugten Subinfektionen. Zeits. Pflanzenkrk. 41: 228-240. 1931.
90. LINFORD, M. B. Studies of pathogenesis and resistance in pea wilt caused by *Fusarium orthoceras* var. *psi*. Phytopath. 21: 797-826. 1931.
91. LINK, K. P. AND WALKER, J. C. The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. Jour. Biol. Chem. 100: 379-383. 1933.
92. LOWIG, E. Ueber den Einfluss des K-ions und der Kalisalz-anionen auf die Widerstandsfähigkeit der Getreidearten gegen den Befall von *Erysiphe graminis*. Ernähr. der Pflanze. 29: 161-165. 1933.
93. LUZ, G. Ueber den Stoffwechsel von *Fusarium lycopersici* und *F. lini*. Phytopath. Zeits. 7: 584-638. 1934.
94. MASSEE, G. On the origin of parasitism in fungi. Phil. Trans. Roy. Soc. Lond. B. 197: 7-24. 1905.
95. MAY, C. The effect of grafting on resistance and susceptibility of tomatoes to *Fusarium* wilt. Phytopath. 20: 519-521. 1930.
96. MELANDER, L. W. AND CRAIGIE, J. H. Nature of resistance of *Berberis* spp. to *Puccinia graminis*. Phytopath. 17: 95-114. 1927.
97. MENON, K. P. V. Studies in the physiology of parasitism. XIV. Comparison of enzymic extracts obtained from various parasitic fungi. Ann. Bot. 48: 187-210. 1934.
98. MIYOSHI, M. Ueber Chemotropismus der Pilze. Bot. Zeit. 52: 1-28. 1894.
99. ———. Die Durchbohrung von Membranen durch Pilzfäden. Jahrb. Wiss. Bot. 28: 269-289. 1895.
100. NAGAI, I. AND IMAMURA, A. Morphology of the neck of the panicle as related to the resistance against blast diseases in rice varieties. (Abs.) Jap. Jour. Bot. 5: 101-102. 1931.
101. NAPPER, M. E. Observations on potato blight (*Phytophthora infestans*) in relation to weather conditions. Jour. Pomol. & Hort. Sci. 11: 177-184. 1933.
102. NEAL, D. C. Cotton wilt: a pathological and physiological investigation. Ann. Mo. Bot. Gard. 14: 359-424. 1927.
103. NEWTON, M. AND BROWN, A. M. Studies on the nature of disease resistance in cereals. I. The reactions to rust of mature and immature tissues. Canad. Jour. Res. 11: 564-581. 1934.
104. NEWTON, R., LEHMANN, J. V. AND CLARKE, A. E. Studies on the nature of rust resistance in wheat. Canad. Jour. Res. 1: 5-35. 1929.
105. NEWTON, R. AND ANDERSON, J. A. Studies on the nature of rust resistance in wheat. IV. Phenolic compounds of the wheat plant. Canad. Jour. Res. 1: 86-99. 1929.
106. NORDHAUSEN, M. Beiträge zur Biologie parasitärer Pilze. Jahrb. Wiss. Bot. 33: 1-46. 1899.
107. PAUL, W. R. C. A comparative morphological and physiological study of a number of strains of *Botrytis cinerea* Pers. with special reference to their virulence. Trans. Brit. Myc. Soc. 14: 118-135. 1929.
108. PEARSON, N. L. Parasitism of *Gibberella saubinetii* on corn seedlings. Jour. Agr. Res. 43: 569-596. 1931.
109. PFEFFER, W. Locomotorische Richtungsbewegungen durch chemische Reize. Unters. Bot. Inst. Tübingen 3: 363-482. 1884.
110. PIERSTORFF, A. L. Studies on the fire-blight organism, *Bacillus amylovorus*. Cornell Agr. Exp. Sta. Mem. 136: 53 pp. 1931.
111. POOL, V. W. AND MCKAY, M. B. Relation of stomatal movement to infection by *Cercospora beticola*. Jour. Agr. Res. 5: 1011-1038. 1916.

112. RADULESCU, E. Beiträge zur Kenntnis der Feldresistenz des Weizens gegen *Puccinia glumarum tritici*. *Planta* 20: 244-286. 1933.
113. REYNOLDS, E. S. Studies on the physiology of plant disease. *Ann. Mo. Bot. Gard.* 18: 57-95. 1931.
114. RICE, M. A. The cytology of host-parasite relations. *Bot. Rev.* 1: 327-353. 1935.
115. ROACH, W. A. Immunity of potato varieties from attack by the wart disease fungus, *Synchytrium endobioticum* (Schilb.) Perc. *Ann. Appl. Biol.* 14: 181-192. 1927.
116. ROCHLIN, E. J. On the question of the non-susceptibility of Cruciferae to *Plasmodiophora brassicae* Wor. Abs. in *Rev. Appl. Myc.* 13: 140-141. 1934.
117. ROSEN, H. R. Efforts to determine the means by which the cotton-wilt fungus, *Fusarium vasinfectum*, produces wilting. *Jour. Agr. Res.* 33: 1143-1162. 1926.
118. SCHAFFNIT, E. AND LÜDTKE, M. Ueber die Bildung von Toxinen durch verschiedene Pflanzenparasiten. *Ber. Deut. Bot. Ges.* 1: 444-463. 1932.
119. ——— AND VOLK, A. Ueber den Einfluss der Ernährung auf die Empfänglichkeit der Pflanzen für Parasiten. *Forsch. Gebiet Pflanzenk.* 3: 1-45. 1927.
120. SCHMIDT, M. Zur Entwicklungsphysiologie von *Cladosporium fulvum* und über die Widerstandsfähigkeit von *Solanum racemigerum* gegen diesen Parasiten. *Planta* 20: 407-439. 1933.
121. SHAW, L. Studies on resistance of apple and Rosaceous plants to fire-blight. *Jour. Agr. Res.* 49: 283-313. 1934.
122. SLEETH, B. *Fusarium nivum*, the cause of watermelon wilt. *W. Va. Agr. Exp. Sta. Bull.* 257: 23 pp. 1934.
123. SMITH, E. F. Wilt disease of cotton, watermelon and cowpea. *U. S. Dept. Agr. Div. Veg. Phys. & Path. Bull.* 17: 74 pp. 1899.
124. SMITH, R. E. Parasitism of *Botrytis cinerea*. *Bot. Gaz.* 33: 421-436. 1902.
125. SPINKS, G. T. Factors affecting susceptibility to disease in plants. *Jour. Agr. Sci.* 5: 231-247. 1913.
126. TAPKE, V. F. Influence of varietal resistance, sap acidity, and certain environmental factors on the occurrence of loose smut in wheat. *Jour. Agr. Res.* 39: 313-339. 1929.
127. THIMANN, K. V. Growth-substances in plants. *Ann. Rev. Biochem.* 4: 545-568. 1935.
128. THOMAS, H. E. Studies on *Armillaria mellea* (Vahl) Quél., infection, parasitism, and host resistance. *Jour. Agr. Res.* 48: 187-218. 1934.
129. THORNTON, H. G. The influence of the host plant in inducing parasitism in lucerne and clover nodules. *Proc. Roy. Soc. Lond. B.* 106: 110-122. 1930.
130. TISDALE, W. H. Flax wilt: a study of the nature and inheritance of wilt resistance. *Jour. Agr. Res.* 11: 573-605. 1917.
131. TOCHINAI, Y. Comparative studies on the physiology of *Fusarium lini* and *Colletotrichum lini*. *Jour. Coll. Agr. Hokkaido Univ.* 14: 171-236. 1926.
132. VALLEAN, W. D. Varietal resistance of plums to brown rot. *Jour. Agr. Res.* 5: 365-396. 1915.
133. VAN DER MEER, J. H. H. Influence of the degree of humidity in the soil on the slime disease caused by *Bacterium solanacearum*. Abs. in *Rev. Appl. Myc.* 9: 210-211. 1930.
134. VAN DER VEEN, R. Investigations on tracheomycoses. Abs. in *Rev. Appl. Myc.* 9: 546-547. 1930.
135. VAN HALL, C. J. J. Das Faulen der jungen Schösslinge und Rhizome von *Iris florentina* und *Iris germanica*, verursacht durch *Bacillus*

- omnivorus* v. Hall und durch einige andere Bakterienarten. Zeits. Pflanzenk. 13: 129-144. 1903.
136. VASUDEVA, R. S. Studies in the physiology of parasitism. XI. An analysis of the factors underlying specialization of parasitism, with special reference to the fungi *Botrytis allii* Munn and *Monilia fructigena* Pers. Ann. Bot. 44: 469-493. 1930.
137. VOLK, A. Beiträge zur Kenntnis der Wechselbeziehungen zwischen Kulturpflanzen, ihren Parasiten und der Umwelt, IV. Phytopath. Zeits. 3: 1-88. 1931.
138. WARD, H. M. On a lily disease. Ann. Bot. 2: 319-382. 1888-9.
139. ———. On predisposition and immunity. Proc. Cambridge Phil. Soc. 11: 307-328. 1902.
140. WATERS, C. W. The reactions of bean rust grown on leaves in solutions. Papers Mich. Acad. Sci. Arts & Letters. 5: 163-177. 1926.
141. WHITE, R. P. Studies on tomato wilt caused by *Fusarium lycopersici* Sacc. Jour. Agr. Res. 34: 197-239. 1927.
142. WICKENS, G. M. Wilt, stem rot, and dieback of the perpetual flowering carnation. Ann. Appl. Biol. 22: 630-683. 1935.
143. WILHELM, P. Studien zur Spezialisierungsweise des Weizengelbrostes, *Puccinia glumarum* f. sp. *tritici* (Schmidt) Erikss. und Henn. und zur Keimungsphysiologie seiner Uredosporen. Arb. Biol. Reichsanst. Land- u. Forstw. 19: 95-133. 1931.
144. WILLAMAN, J. J. Biochemistry of plant diseases. VII. Correlation between skin texture and flesh texture in plum varieties. Proc. Soc. Exp. Biol. & Med. 23: 680-681. 1926.
145. WILLISON, R. S. Wound-gum in peaches and grapes. Its relation to the invasion of fungus wound-parasites. Sci. Agr. 12: 402-419, 484-505. 1932.
146. WILTSHIRE, S. P. Infection and immunity studies on the apple and pear scab fungi. Ann. Appl. Biol. 1: 335-349. 1915.
147. WORMALD, H. The brown rot diseases of fruit trees. Min. Agr. & Fish. Bull. 88: 50 pp. 1935.
148. YARWOOD, C. E. The comparative behaviour of four clover-leaf parasites on excised leaves. Phytopath. 24: 797-806. 1934.
149. YOUNG, P. A. Facultative parasitism and host ranges of fungi. Amer. Jour. Bot. 13: 502-520. 1926.

